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(54) Title: AMINIMIDE-CONTAINING MOLECULES AND MATERIALS AS MOLECULAR RECOGNITION AGENTS

(57) Abstract

The design and synthesis of novel aminimide-based molecular modules and the use of the modules in the construction of new molecules and fabricated materials is disclosed. The new molecules and fabricated materials are molecular recognition agents useful in the design and synthesis of drugs, and have applications in separations and materials science.

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## AMINIMIDE-CONTAINING MOLECULES AND
## MATERIALS AS MOLECULAR RECOGNITION AGENTS

### 1.  FIELD OF THE INVENTION

The present invention relates to the *logical*
development of biochemical and biopharmaceutical agents
and of new materials including fabricated materials such
as fibers, beads, films, and gels.  Specifically, the
invention relates to the development of molecular modules
based on aminimide and related structures, and to the use
of these modules in the assembly of molecules and
fabricated materials with tailored properties, which are
determined by the contributions of the individual
building modules.  The molecular modules of the invention
are preferably chiral, and can be used to synthesize new
compounds and fabricated materials which are able to
recognize biological receptors, enzymes, genetic
materials, and other chiral molecules, and are thus of
great interest in the fields of biopharmaceuticals,
separation and materials science.

### 2.  BACKGROUND OF THE INVENTION

The discovery of new molecules has
traditionally focused in two broad areas, biologically
active molecules, which are used as drugs for the
treatment of life-threatening diseases, and new
materials, which are used in commercial, especially high-
technological applications.  In both areas, the strategy
used to discover new molecules has involved two basic
operations: (i) a more or less random choice of a
molecular candidate, prepared either via chemical
synthesis or isolated from natural sources, and (ii) the
testing of the molecular candidate for the property or
properties of interest.  This discovery cycle is repeated
indefinitely until a molecule possessing the desirable
properties is located.  In the majority of cases, the

molecular types chosen for testing have belonged to
rather narrowly defined chemical classes. For example,
the discovery of new peptide hormones has involved work
with peptides; the discovery of new therapeutic steroids
has involved work with the steroid nucleus; the discovery
5   of new surfaces to be used in the construction of
computer chips or sensors has involved work with
inorganic materials, etc. As a result, the discovery of
new functional molecules, being *ad hoc* in nature and
relying predominantly on serendipity, has been an
10  extremely time-consuming, laborious, unpredictable, and
costly enterprise.

A brief account of the strategies and tactics
used in the discovery of new molecules is described
below. The emphasis is on biologically interesting
15  molecules; however, the technical problems encountered
in the discovery of biologically active molecules as
outlined here are also illustrative of the problems
encountered in the discovery of molecules which can serve
as new materials for high technological applications.
20  Furthermore, as discussed below, these problems are also
illustrative of the problems encountered in the
development of fabricated materials for high
technological applications.

25  2.1  Drug Design

Modern theories of biological activity state
that biological activities, and therefore physiological
states, are the result of molecular recognition events.
For example, nucleotides can form complementary base
30  pairs so that complementary single-stranded molecules
hybridize resulting in double- or triple-helical
structures that appear to be involved in regulation of
gene expression. In another example, a biologically
active molecule, referred to as a ligand, binds with
35  another molecule, usually a macromol cule referred to as

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ligand-acceptor (e.g. a receptor or an enzyme), and this
binding elicits a chain of molecular events which
ultimately gives rise to a physiological state, e.g.
normal cell growth and differentiation, abnormal cell·
growth leading to carcinogenesis, blood-pressure
5   regulation, nerve-impulse-generation and -propagation,
etc.  The binding between ligand and ligand-acceptor is
geometrically characteristic and extraordinarily
specific, involving appropriate three-dimensional
structural arrangements and chemical interactions.
10

2.1.1    Design and Synthesis of Nucleotides
Recent interest in gene therapy and
manipulation of gene expression has focused on the design
of synthetic oligonucleotides that can be used to block
15  or suppress gene expression via an antisense, ribozyme or
triple helix mechanism.  To this end, the sequence of the
native target DNA or RNA molecule is characterized and
standard methods are used to synthesize oligonucleotides
representing the complement of the desired target
20  sequence (see, S. Crooke, The FASEB Journal, Vol. 7, Apr.
1993, p. 533 and references cited therein).  Attempts to
design more stable forms of such oligonucleotides for use
in vivo have typically involved the addition of various
functional groups, e.g., halogens, azido, nitro, methyl,
25  keto, etc. to various positions of the ribose or
deoxyribose subunits (cf., The Organic Chemistry of
Nucleic Acids, Y. Mizuno, Elsevier Science Publishers BV,
Amsterdam, The Netherlands, 1987).

30  2.1.2    Glycopeptides
As a result of recent advances in biological
carbohydrate chemistry, carbohydrates increasingly are
being viewed as the components of living systems with the
enorm usly complex structures required for the encoding
35  of the massive amounts of information needed to

orchestrate the processes of life, e.g., cellular recognition, immunity, embryonic development, carcinogenesis and cell-death. Thus, whereas two naturally occurring amino acids can be used by nature to convey 2 fundamental molecular messages, i.e., via formation of the two possible dipeptide structures, and four different nucleotides convey 24 molecular messages, two different monosaccharide subunits can give rise to 11 unique disaccharides, and four dissimilar monosaccharides can give rise to up to 35,560 unique tetramers each capable of functioning as a fundamental discreet molecular messenger in a given physiological system.

The gangliosides are examples of the versatility and effect with which organisms can use saccharide structures. These molecules are glycolipids (sugar-lipid composites) and as such are able to position themselves at strategic locations on the cell wall: their lipid component enables them to anchor in the hydrophobic interior of the cell wall, positioning their hydrophilic component in the aqueous extracellular millieu. Thus the gangliosides (like many other saccharides) have been chosen to act as cellular sentries: they are involved in both the inactivation of bacterial toxins and in contact inhibition, the latter being the complex and poorly understood process by which normal cells inhibit the growth of adjacent cells, a property lost in most tumor cells. The structure of ganglioside GM, a potent inhibitor of the toxin secreted by the cholera organism, featuring a branched complex pentameric structure is shown below.
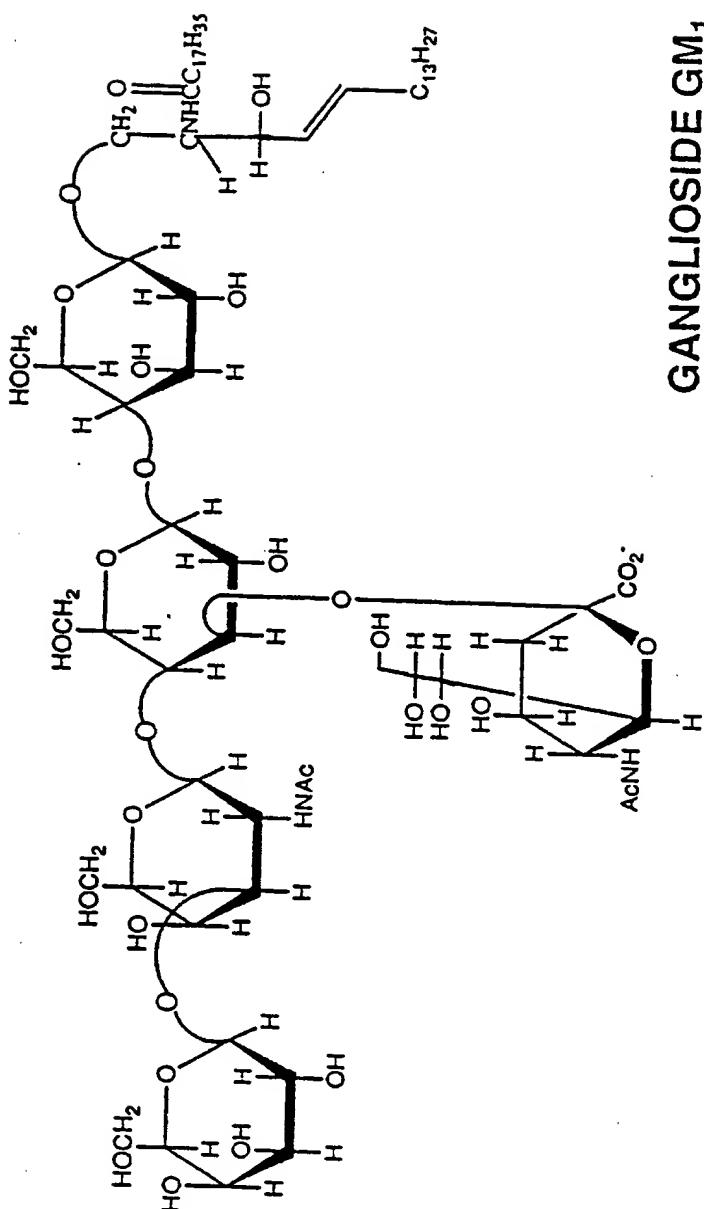
GANGLIOSIDE GM₁

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The oligosaccharide components of the glycoproteins (sugar-protein composites) responsible for the human blood-group antigens (the A, B, and O blood classes) are shown below.

5

10
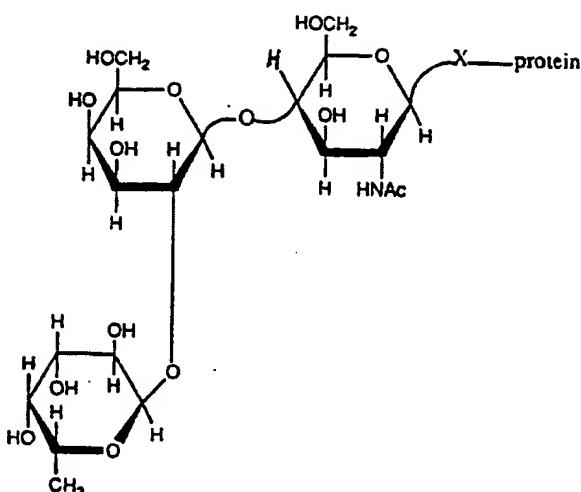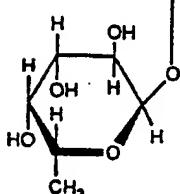


**BLOOD GROUP O ANTIGEN, TYPE II**

15

20

25
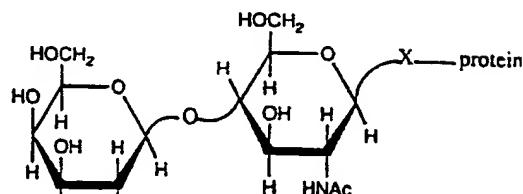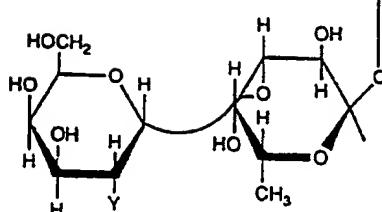


Blood group a: Y=NHA$_c$
Blood group b: Y=OH

30

35                          **BLOOD GROUP A AND B ANTIGENS**

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Interactions involving complementary proteins and glycoproteins on red blood cells belonging to incompatible blood classes cause formation of aggregates, or clusters and are the cause for failed transfusions of human blood.

Numerous other biological processes and macromolecules are controlled by glycosylation (i.e., the covalent linking with sugars). Thus, deglycosylation of erythropoetin causes loss of the hormone's biological activity; deglycosylation of human gonadotropic hormone increases receptor binding but results in almost complete loss of biological activity (see Rademacher et al., Ann. Rev. Biochem 57, 785 (1988); and glycosylation of three sites in tissue plasminogen activating factor (TPA) produces a glycopolypeptide which is 30% more active than the polypeptide that has been glycosylated at two of the sites.

2.1.3    Design and Synthesis of Mimetics
         of Biological Ligands

A currently favored strategy for development of agents which can be used to treat diseases involves the discovery of forms of ligands of biological receptors, enzymes, or related macromolecules, which mimic such ligands and either boost, i.e., agonize, or suppress, i.e., antagonize the activity of the ligand. The discovery of such desirable ligand forms has traditionally been carried out either by random screening of molecules (produced through chemical synthesis or isolated from natural sources), or by using a so-called "rational" approach involving identification of a lead-structure, usually the structure of the native ligand, and optimization of its properties through numerous cycles of structural redesign and biological testing. Since most useful drugs have been disc vered not through the "rational" approach but through the screening of

rand mly chosen compounds, a hybrid approach to drug
discovery has recently emerged which is based on the use
of combinatorial chemistry to construct huge libraries of
randomly-built chemical structures which are screened for
specific biological activities.  (S. Brenner and R.A.
5    Lerner, 1992, Proc. Natl. Acad. Sci. USA 89:5381)

          Most lead-structures which have been used in
"rational" drug design are native polypeptide ligands of
receptors or enzymes.  The majority of polypeptide
ligands, especially the small ones, are relatively
10   unstable in physiological fluids, due to the tendency of
the peptide bond to undergo facile hydrolysis in acidic
media or in the presence of peptidases.  Thus, such
ligands are decisively inferior in a pharmacokinetic
sense to nonpeptidic compounds, and are not favored as
15   drugs.  An additional limitation of small peptides as
drugs is their low affinity for ligand acceptors.  This
phenomenon is in sharp contrast to the affinity
demonstrated by large, folded polypeptides, e.g.
proteins, for specific acceptors, e.g. receptors or
20   enzymes, which is in the subnanomolar range.  For
peptides to become effective drugs, they must be
transformed into nonpeptidic organic structures, i.e.,
peptide mimetics, which bind tightly, preferably in the
nanomolar range, and can withstand the chemical and
25   biochemical rigors of coexistence with biological fluids.

          Despite numerous incremental advances in the
art of peptidomimetic design, no general solution to the
problem of converting a polypeptide-ligand structure to a
peptidomimetic has been defined.  At present, "rational"
30   peptidomimetic design is done on an ad hoc basis.  Using
numerous redesign-synthesis-screening cycles, peptidic
ligands belonging to a certain biochemical class have
been converted by groups of organic chemists and
pharmacologists to specific peptidomimetics; however, in
35   the majority  f cases the results in one biochemical

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area, e.g. peptidas  inhibitor design using the enzyme substrate as a lead cannot be transferred for use in another area, e.g. tyrosine-kinase inhibitor design using the kinase substrate as a lead.

5  In many cases, the peptidomimetics that result from a peptide structural lead using the "rational" approach comprise unnatural α-amino acids.  Many of these mimetics exhibit several of the troublesome features of native peptides (which also comprise α-amino acids) and are, thus, not favored for use as drugs.  Recently,
10 fundamental research on the use of nonpeptidic scaffolds, such as steroidal or sugar structures, to anchor specific receptor-binding groups in fixed geometric relationships have been described (see for example Hirschmann, R. et al., 1992 J. Am. Chem. Soc., 114:9699-9701; Hirschmann,
15 R. et al., 1992 J. Am. Chem. Soc., 114:9217-9218); however, the success of this approach remains to be seen.

In an attempt to accelerate the identification of lead-structures, and also the identification of useful drug candidates through screening of randomly chosen
20 compounds, researchers have developed automated methods for the generation of large combinatorial libraries of peptides and certain types of peptide mimetics, called "peptoids", which are screened for a desirable biological activity.  For example, the method of H. M. Geysen, (1984
25 Proc. Natl. Acad. Sci. USA 81:3998) employs a modification of Merrifield peptide synthesis wherein the C-terminal amino acid residues of the peptides to be synthesized are linked to solid-support particles shaped as polyethylene pins; these pins are treated individually
30 or collectively in sequence to introduce additional amino-acid residues forming the desired peptides.  The peptides are then screened for activity without removing them from the pins.  Houghton, (1985, Proc. Natl. Acad. Sci. USA 82:5131; and U.S. Patent No. 4,631,211) utilizes
35 individual polyethylene bags ("tea bags") containing

C-terminal amino acids bound to a solid support. These
are mixed and coupled with the requisite amino acids
using solid phase synthesis techniques. The peptides
produced are then recovered and tested individually.
Fodor et al., (1991, Science 251:767) described light-
directed, spatially addressable parallel-peptide
synthesis on a silicon wafer to generate large arrays of
addressable peptides that can be directly tested for
binding to biological targets. These workers have also
developed recombinant DNA/genetic engineering methods for
expressing huge peptide libraries on the surface of
phages (Cwirla et al., 1990, Proc. Natl. Acad. Sci. USA
87:6378).

In another combinatorial approach, V. D.
Huebner and D.V. Santi (U.S. Patent No. 5,182,366)
utilized functionalized polystyrene beads divided into
portions each of which was acylated with a desired amino
acid; the bead portions were mixed together and then
split into portions each of which was subjected to
acylation with a second desirable amino acid producing
dipeptides, using the techniques of solid phase peptide
synthesis. By using this synthetic scheme, exponentially
increasing numbers of peptides were produced in uniform
amounts which were then separately screened for a
biological activity of interest.

Zuckerman et al., (1992, Int. J. Peptide
Protein Res. 91:1) also have developed similar methods
for the synthesis of peptide libraries and applied these
methods to the automation of a modular synthetic
chemistry for the production of libraries of N-alkyl
glycine peptide derivatives, called "peptoids", which are
screened for activity against a variety of biochemical
targets. (See also, Symon et al., 1992, Proc. Natl.
Acad. Sci. USA 89:9367). Encoded combinatorial chemical
syntheses have been described recently (S. Brenner and
R.A. Lerner, 1992, Proc. Natl. Acad. Sci. USA 89:5381).

In additi n to the lead structure, a very useful source of information for the realization of the preferred "rational" drug discovery is the structure of the biological ligand acceptor which, often in conjunction with molecular modelling calculations, is used to simulate modes of binding of the ligand with its acceptor; information on the mode of binding is useful in optimizing the binding properties of the lead-structure. However, finding the structure of the ligand acceptor, or preferably the structure of a complex of the acceptor with a high affinity ligand, requires the isolation of the acceptor or complex in the pure, crystalline state, followed by x-ray crystallographic analysis. The isolation and purification of biological receptors, enzymes, and the polypeptide substrates thereof are time-consuming, laborious, and expensive; success in this important area of biological chemistry depends on the effective utilization of sophisticated separation technologies.

Crystallization can be valuable as a separation technique but in the majority of cases, especially in cases involving isolation of a biomolecule from a complex biological *milieu*, successful separation is chromatographic. Chromatographic separations are the result of reversible differential binding of the components of a mixture as the mixture moves on an active natural, synthetic, or semisynthetic surface; tight-binding components in the moving mixture leave the surface last *en masse* resulting in separation.

The development of substrates or supports to be used in separations has involved either the polymerization/crosslinking of monomeric molecules under various conditions to produce fabricated materials such as beads, gels, or films, or the chemical modification of various commercially available fabricated materials e.g., sulfonation  f polystyrene beads, to produce the desired

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new materials.  In the majority of cases, prior art
support materials have been developed to perform specific
separations or types of separations and are thus of
limited utility.  Many of these materials are
incompatible with biological macromolecules, e.g.,
5    reverse-phase silica frequently used to perform high
pressure liquid chromatography can denature hydrophobic
proteins and other polypeptides.  Furthermore, many
supports are used under conditions which are not
compatible with sensitive biomolecules, such as proteins,
10   enzymes, glycoproteins, etc., which are readily
denaturable and sensitive to extreme pH's.  An additional
difficulty with separations carried out using these
supports is that the separation results are often
support-batch dependent, i.e. they are irreproducible.
15         Recently a variety of coatings and composite-
forming materials have been used to modify commercially
available fabricated materials into articles with
improved properties; however the success of this approach
remains to be seen.
20         If a chromatographic support is equipped with
molecules which bind specifically with a component of a
complex mixture, that component will be separated from
the mixture and may be released subsequently by changing
the experimental conditions (e.g. buffers, stringency,
25   etc.)  This type of separation is appropriately called
affinity chromatography and remains an extremely
effective and widely used separation technique.  It is
certainly much more selective than traditional
chromatographic techniques, e.g chromatography on silica,
30   alumina, silica or alumina coated with long-chain
hydrocarbons, polysaccharide and other types of beads or
gels which in order to attain their maximum separating
efficiency need to be used under conditions that are
damaging to biomolecules,   .g. conditions involving high
35

pressure, use of organic s lv nts and other denaturing
agents, etc.

The development of more powerful separation
technologies depends significantly on breakthroughs in
the field of materials science, specifically in the
5   design and construction of materials that have the power
to recognize specific molecular shapes under experimental
conditions resembling those found in physiological media,
i.e. these experimental conditions must involve an
aqueous medium whose temperature and pH are close to the
10  physiological levels and which contains none of the
agents known to damage or denature biomolecules.  The
construction of these "intelligent" materials frequently
involves the introduction of small molecules capable of
specifically recognizing others into  existing materials,
15  e.g. surfaces, films, gels, beads, etc., by a wide
variety of chemical modifications; alternatively
molecules capable of recognition are converted to
monomers and used to create the "intelligent" materials
through polymerization reactions.
20

3.   SUMMARY

A new approach to the construction of novel
molecules is described.  This approach involves the
development of aminimide-based molecular building blocks,
25  containing appropriate atoms and functional groups, which
may be chiral and which are used in a modular assembly of
molecules with tailored properties; each module
contributing to the overall properties of the assembled
molecule.  The aminimide building blocks of the invention
30  can be used to synthesize novel molecules designed to
mimic the three-dimensional structure and function of
native ligands, and/or interact with the binding sites of
a native receptor.  This *logical* approach to molecular
construction is applicable to the synthesis of all types
35  of molecules, including but not limited to mimetics of

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peptides, proteins, oligonucleotides, carbohydrates, lipids, polymers and to fabricated materials useful in materials science.  It is analogous to the modular construction of a mechanical device that performs a specific operation wherein each module performs a specific task contributing to the overall operation of the device.

The invention is based, in part, on the following insights of the discoverer.  (1) All ligands share a single universal architectural feature:  they consist of a scaffold structure, made e.g. of amide, carbon-carbon, or phosphodiester bonds which support several functional groups in a precise and relatively rigid geometric arrangement.  (2) Binding modes between ligands and receptors share a single universal feature as well:  they all involve attractive interactions between complementary structural elements, e.g., charge- and $\pi$-type interactions, hydrophobic and Van der Waals forces, hydrogen bonds.  (3) A continuum of fabricated materials exists spanning a dimensional range from 100 Å to 1 cm in diameter comprising various materials of varied construction, geometries, morphologies and functions, all of which possess the common feature of a functional surface which is presented to a biologically active molecule or a mixture of molecules to achieve recognition between the molecule (or the desired molecule in a mixture) and the surface.  And (4) Aminimide structures, which have remained relatively unexplored in the design and synthesis of biologically active compounds and especially of drugs, would be ideal building blocks for constructing backbones or scaffolds bearing the appropriate functional groups, that either mimic desired ligands and/or interact with appropriate receptor binding sites; furthermore, aminimide modules may be utilized in a variety of ways across the continuum of fabricated materials described above to produce new materials

capable of specific molecular recognition. These aminimide building blocks may be chirally pure and can be used to synthesize molecules that mimic a number of biologically active molecules, including but not limited to peptides, proteins, oligonucleotides, polynucleotides, carbohydrates, lipids, and a variety of polymers and fabricated materials that are useful as new materials, including but not limited to solid supports useful in column chromatography, catalysts, solid phase immunoassays, drug delivery vehicles, films, and "intelligent" materials designed for use in selective separations of various components of complex mixtures.

Working examples describing the use of aminimide-based modules in the modular assembly of a variety of molecular structures are given. The molecular structures include functionalized silica surfaces useful in the optical resolution of racemic mixtures; peptide mimetics which inhibit human elastase, protein-kinase, and the HIV protease; polymers formed via free-radical or condensation polymerization of aminimide-containing monomers; and lipid-mimetics useful in the detection, isolation, and purification of a variety of receptors.

In accordance with the objectives of the present invention, the aminimide-based molecules of interest possess the desired stereochemistry and, when required, are obtained optically pure. In addition to the synthesis of single molecular entities, the synthesis of libraries of aminimide-based molecules, using the techniques described herein or modifications thereof which are well known in the art to perform combinatorial chemistry, is also within the scope of the invention. Furthermore, the aminimide-containing molecules possess enhanced hydrolytic and enzymatic stabilities, and in the case of biologically active materials, are transported to target ligand-acceptor macromolecules *in vivo* without causing any seri us side-effects.

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4.    DETAILED DESCRIPTION OF THE INVENTION

              To the extent necessary to further understand
        any portion of the detailed description, the following
        earlier filed U.S. patent applications are expressly
        incorporated herein by reference thereto:   AMINIMIDE
5       COMPOSITIONS AND BIOLOGICALLY USEFUL DERIVATIVES THEREOF,
        Serial No. 07/906,770, filed June 30, 1992; AMINIMIDE-
        BASED SUPPORT MATERIALS AND FUNCTIONALIZED SURFACES,
        Serial No. 07/906,769, filed June 30, 1992; DIRECTED PURE
        CHIRAL-ISOMER LIGANDS, RECOGNITION AGENTS AND
10      FUNCTIONALLY USEFUL MATERIALS FROM SUBSTITUTED AMINIMIDES
        AND DERIVATIVES CONTAINING AN ASYMMETRIC CENTER, Serial
        No. 08/041,559, filed April 2, 1993.


15


        4.1    Physical and Chemical Properties of the
               Aminimide Functional Group

              Aminimides are zwitterionic structures
20      described by the resonance hybrid of the two
        energetically comparable Lewis structures shown below.
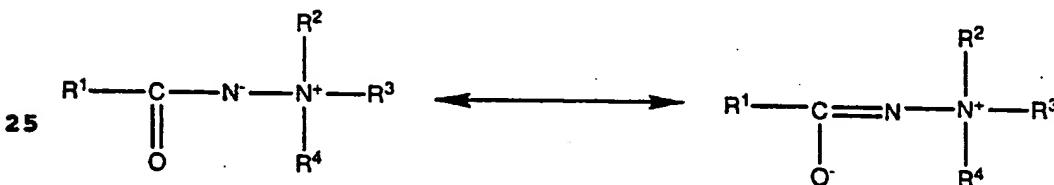


              The tetrasubstituted nitrogen of the aminimide
30      group can be asymetric rendering aminimides chiral as
        shown by the two enantiomers below.

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As a result of the polarity of their structures, but lack of net charge, simple aminimides are freely soluble in both water and (especially) organic solvents.

Dilute aqueous solutions of aminimides are neutral and of very low conductivity; aminimide conjugate acids are weakly acidic, $pK_a \cong 4.5$. A striking property of aminimides is their hydrolytic stability, under acidic, basic, or enzymatic conditions. For example, boiling trimethyl amine benzamide in 6 N NaOH for 24 hrs leaves the aminimide unchanged. Upon thermolytic treatment, at temperatures exceeding 180°C, aminimides decompose to give isocyanates as follows.

$$R_1-\underset{\underset{O^-}{|}}{C}=N-\underset{\underset{R_3}{|}}{\overset{\overset{R_2}{|}}{N^+}}-R_4 \longrightarrow R_1-N=C=O + \underset{\underset{R_3}{|}}{\overset{\overset{R_2}{|}}{N}}-R_4$$

**4.1.1    Use of the Aminimide Group as a
          Mimetic of the Amide Group**

The aminimide group mimics several key structural features of the amide group, such as overall geometry (e.g. both functional groups contain a planar carbonyl unit and a tetrahedral atom linked to the acylated nitrogen) and aspects of charge distribution (e.g. both functional groups contain a carbonyl with significant negative charge development on the oxygen). These structural relationships can be seen below, where the resonance hybrids of the two groups are drawn.

5

$$\underset{}{\overset{O}{\underset{}{\parallel}}}\;\;\overset{R}{\underset{}{\underset{}{\overset{\text{'''}}{C}}}}\overset{H}{\underset{}{}}\qquad\longleftrightarrow\qquad \overset{O^{-}}{\underset{}{}}\;\;\overset{R}{\underset{}{\overset{\text{'''}}{C}}}\overset{H}{\underset{}{}}$$
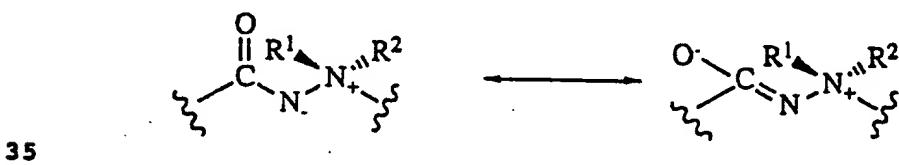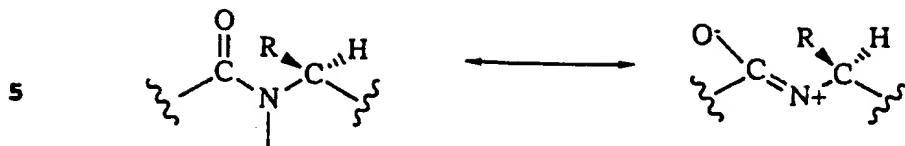
10          Being hydrolytically and enzymatically more
stable than amides and possessing novel solubility
properties due to their zwitterionic structures,
aminimides are valuable building blocks for the
construction of mimetics of biologically active molecules
15  with superior pharmacological properties.  For the
construction of these mimetics, the aminimide backbone is
used as a scaffold for the geometrically precise
attachment of structural units possessing desired
stereochemical and electronic features, such as suitable
20  chiral atoms, hydrogen-bonding centers, hydrophobic and
charged groups, $\pi$-systems, etc.  Furthermore, multiple
aminimide units can be linked in a variety of modes,
using likers of diverse structures, to produce polymers
of a great variety of structures.  Specific molecular
25  forms are chosen for screening and further study using
several criteria.  In one instance a certain aminimide
structure is chosen because it is novel and has never
been tested for activity as a biopharmaceutical agent or
as material for device construction.  In a preferable
30  instance an aminimide ligand is chosen because it
incorporates structural features and properties suggested
by a certain biochemical mechanism.  In another
preferable case the aminimide structure is the result of
assembly of molecular modules each making a specific
35

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desirable contribution to the overall properties of the aminimide-containing molecule.

Summarizing, aminimides are functional groups with unusual and very desirable physiochemical properties, which can be used as molecular modules for the construction of molecular structures that are useful as biopharmaceutical agents and as new materials for high technological applications.

**4.2   General Synthetic Routes to Aminimides**

**4.2.1      Aminimides via Alkylation of N,N-Disubstituted Hydrazones**

Alkylation of an acyl hydrazide (hydrazone) followed by neutralization with a base produces an aminimide.

$$R^1{-}\underset{R^2}{\overset{}{N}}{-}\underset{H}{\overset{}{N}}{-}\overset{\overset{O}{\|}}{C}{-}R^4 \quad \xrightarrow[\text{(2) neutralization}]{\text{(1) } R^3X} \quad R^1{-}\underset{R^2}{\overset{+}{N}}{-}\underset{N^-}{\overset{R^3}{N}}{-}\overset{\overset{O}{\|}}{C}{-}R^4$$
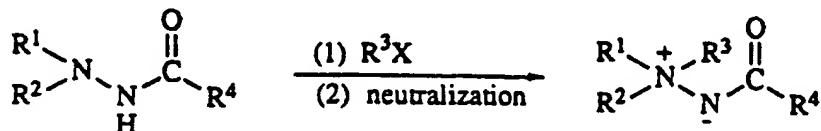
This alkylation is carried out in a suitable solvent such as a hydroxylic solvent e.g. water, ethanol, isopropanol or a dipolar aprotic solvent e.g., DMF, DMSO, acetonitrile, usually with heating.

The acyl hydrazide is produced by the reaction of a 1,1-disubstituted hydrazine with an activated acyl derivative or an isocyanate, in a suitable organic solvent, e.g. methylene chloride, toluene, ether, etc. in the presence of a base such as triethylamine to neutralize the haloacid generated during the acylation.

$$R^1{-}\underset{R^2}{\overset{}{N}}{-}NH_2 \quad + \quad Cl{-}\overset{\overset{O}{\|}}{C}{-}R^4 \quad \longrightarrow \quad R^1{-}\underset{R^2}{\overset{}{N}}{-}\underset{H}{\overset{}{N}}{-}\overset{\overset{O}{\|}}{C}{-}R^4$$
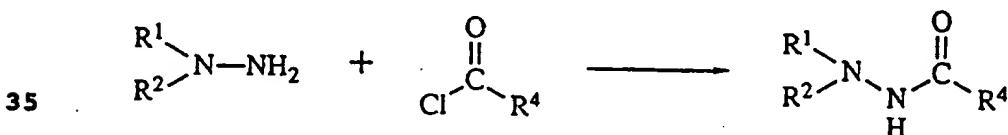
Activated acyl derivatives include acid chlorides, chlorocarbonates, chlorothiocarbonates, etc.; the acyl derivative may also be replaced with a suitable carboxylic acid and a condensing agent such as

5   dicyclohexylcarbodiimide (DCC).

The alkylating agent $R^3X$ used in the hydrazone alkylation may be an alkyl halide (X = Cl, Br, I), a tosylate (X = OTs), or some other suitable reactive species, such as an epoxide. The conversion of phenyl

10  isocyanate to an aminimide using the commercially available 1,1-dimethylhydrazine and ethylene oxide as the hydrazone alkylating agent is given below:

15

20
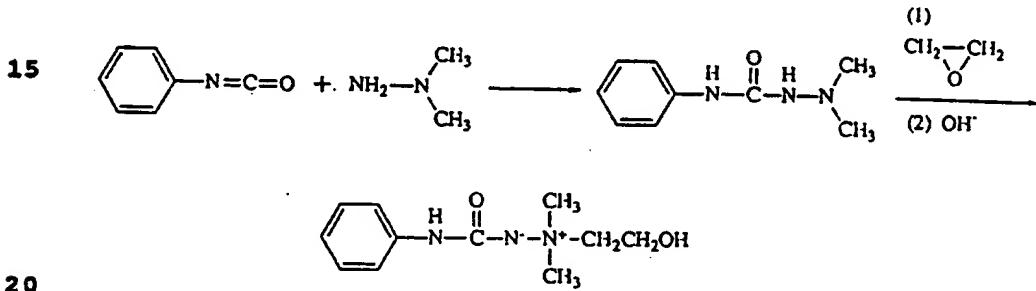
The desired 1,1-disubstituted hydrazines may be readily prepared in a number of ways well known in the art; one is the reaction of a secondary amine with $NH_2Cl$ in an inert organic solvent.

25

30

The above route to aminimides is broadly applicable and allows the incorporation of a wide variety of aliphatic, ar matic and heterocyclic groups into

35  various positions in the aminimide structure.

- 21 -

4.2.2      Aminimides via Acylation of 1,1,1-Trialkyl
           Hydrazinium Salts

           Acylation of a suitable trialkyl hydrazinium
salt by an acyl derivative or isocyanate in the presence
of a strong base in a suitable organic solvent, e.g.
5 dioxane, ether, acetonitrile, etc. produces good yields
of aminimides.

$$
\begin{array}{c} R^2 \\ | \\ R^1{-}N^+{-}NH_2 \; X^- \\ | \\ R^3 \end{array}
\quad + \quad
\begin{array}{c} O \\ \| \\ R^4{-}C{-}OR^5 \end{array}
\quad \xrightarrow{\text{base}} \quad
\begin{array}{cc} R^2 & O \\ | & \| \\ R^1{-}N^+{-}N^-{-}C{-}R^4 \\ | \\ R^3 \end{array}
$$
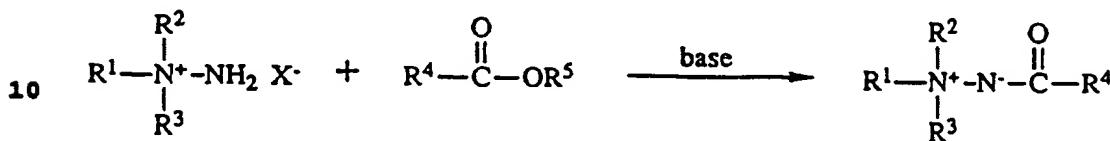
           The acyl derivatives for the acylation reaction are the
same as those required for the synthesis of the
15 hydrazones outlined above.

           The required hydrazinium salts may be prepared
by routine alkylation of a 1,1-disubstituted hydrazines
or by treatment of a tertiary amine with a haloamine (see
78 J. Am. Chem. Soc. 1211 (1956)).

20         Hydrazinium salts, being chiral at nitrogen,
may be resolved, e.g. by treatment with a chiral acid
followed by separation of the diastereomers (e.g. using
chromatography or fractional crystallization and the
resulting enantiomers used in stereoselective syntheses
25 of aminimides.

           When one of the alkyl groups in a hydrazinium
salt is an ester group, the ester may be saponified
efficiently using LiOH in a mixture of methanol and
water, producing a useful α-hydrazinium acid after
30 neutralization of the reaction mixture with an acid.

$$
\begin{array}{c} R^2 \quad R^3 \\ \diagdown \; N^+ \diagup \\ \diagup \quad\quad \diagdown \\ H_2N \quad\quad CH_2 \\ | \\ COOR^1 \end{array}
\quad \begin{array}{c} \text{1. LiOH} \\ \text{MeOH-H}_2\text{O} \\ \hline \text{2. neutralization} \end{array} \quad
\begin{array}{c} R^2 \quad R^3 \\ \diagdown \; N^+ \diagup \\ \diagup \quad\quad \diagdown \\ H_2N \quad\quad CH_2 \\ | \\ COO^- \end{array}
$$

X⁻
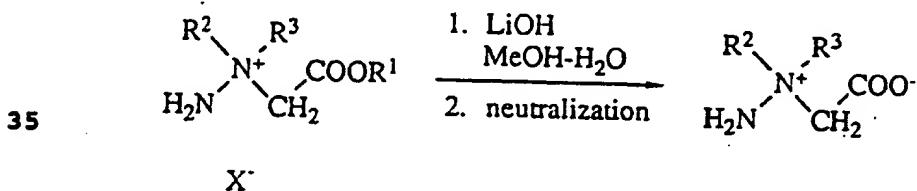
- 22 -

Suitably protected hydrazinium carboxylates may be used
in condensation reactions to produce aminimides.
Procedures analogous to those known to be useful in
effecting peptide bond formation are expected to be
useful; e.g. DCC or other carbodiimides may be used as
condensing agents in solvents such as DMF.
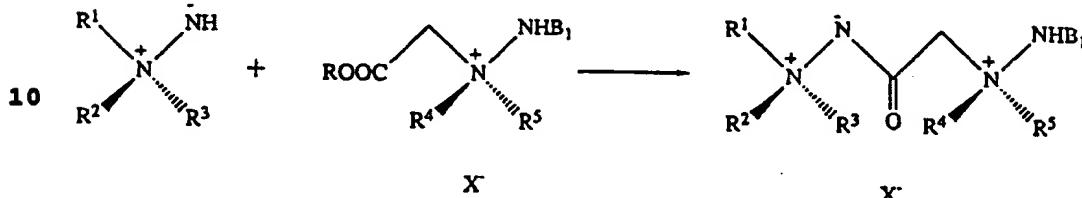
Alternatively, the hydrazinium carboxylate units may be
coupled with α-amino-acids or with other nucleophiles,
such as amines, thiols, alcohols, etc., using standard
techniques, to produce molecules of wide utility as
ligand mimetics and new materials for high technological
applications.

The α-hydrazinium esters may in turn be
produced by the alkylation of a 1,1-disubstituted
hydrazine with a haloester under standard reaction
conditions, such as those given above for the alkylation
of hydrazones.
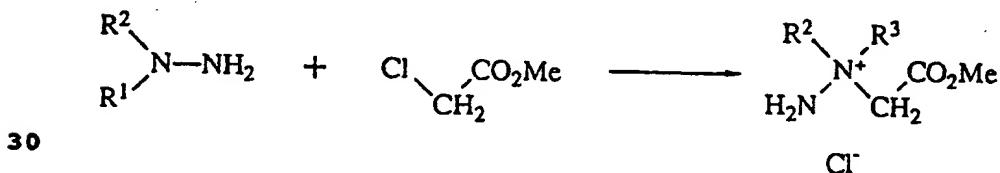
Alternatively, these hydrazinium esters may be produced
by standard alkylation of the appropriate α-hydrazino
ester.

- 23 -
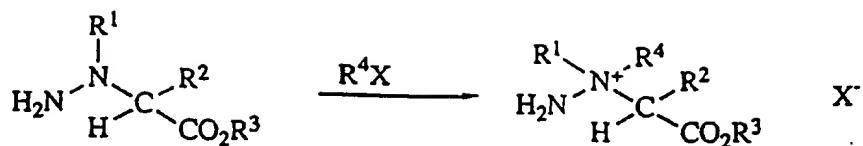
5      The required 1,1-disubstituted hydrazine for
the above reaction may be obtained by acid or base
hydrolysis of the corresponding hydrazone (see 108 J. Am.
Chem. Soc. 6394 (1986)); the alkylated hydrazone is
produced from the monosubstituted hydrazone by the method
10   of Hinman and Flores (24 J. Org. Chem. 660 (1958)).



15

20

25
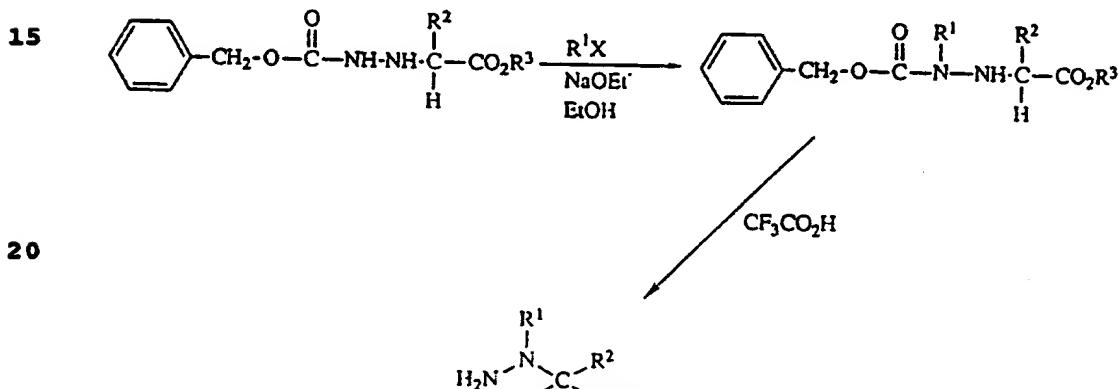
The monosubstituted hydrazones required above may be
obtained by reduction of the Schiff base formed from an
α-keto ester and a suitable hydrazone.  This reduction
30   may also be carried out stereoselectively, if desired,
using DuPHOS-Rhodium catalysis (114 J. Am. Chem. Soc.
6266 (1992); 259 Science 479 (1993)), as shown:

35

- 24 -
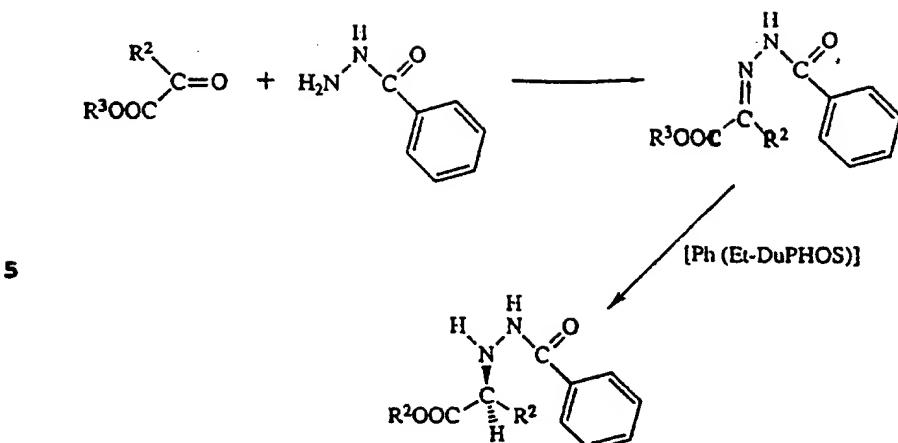


10  In a variation of the synthesis given above, α-halo aminimides are prepared using an α-halo acyl halide.

$$(CH_3)_3N^+NH_2 \; OTs^- \; + \; ClCH_2COCl \longrightarrow (CH_3)_3N^+N^-COCH_2Cl$$

15

These halo aminimides may be reacted with nucleophiles containing reactive hydroxyl, thio, or amino groups to give complex aminimide structures.

20  4.2.3    Aminimides via the Hydrazine-Epoxide-Ester Reaction

A very useful and versatile synthesis of aminimides involves the one-pot reaction of an epoxide, an asymetrically disubstituted hydrazine, and an ester in
25  a hydroxylic solvent, usually water or an alcohol, which is allowed to proceed usually at room temperature over several hours to several days.

30

35

5

10

15

$$R^1-CH-CH_2 \; + \; \underset{R^3}{\overset{R^2}{N}}-NH_2 \; + \; R^4-COOR^5 \longrightarrow$$

$$R^1-CH-CH_2-\underset{R^3}{\overset{R^2}{N^+}}-N'-\underset{O}{\overset{}{C}}-R^4 \; + \; R^5OH$$

20

25

30

35
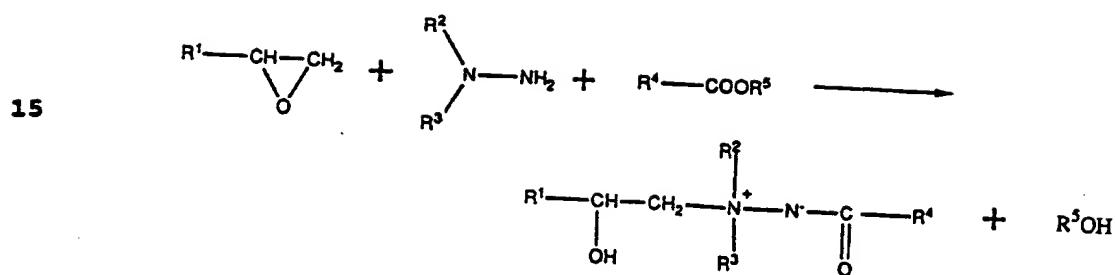
In the equation abov , $R^1$, $R^2$ and $R^3$ are selected from a
set of diverse structural types (e.g. alkyl, cycloalkyl,
aryl, aralkyl, alkaryl or many substituted versions
thereof), and $R^4$ and $R^5$ are alkyl or cycloalkyl.

5    The rates for the above reaction increase with
increasing electrophilicity of the ester component.
Generally, a mixture of 0.1 mol of each of the reactants
in 50-100 ml of an appropriate solvent is stirred for the
required period at room temperature (the reaction may be
monitored by thin layer chromatography). At the end of
10   this period the solvent is removed *in vacuo* to give the
crude product.

If substituent $R^4$ of the ester component in the
above aminimide formation contains a double bond, an
aminimide with a terminal double bond results which may
15   be epoxidized, e.g. using a peracid under standard
reaction conditions, and the resulting epoxide used as
starting material for a new aminimide formation; thus a
structure containing two aminimide subunits results. If
the aminimide-formation and epoxidation sequence is
20   repeated n times, a structure containing n aminimide
subunits results; thus for $R^4$ = propene, n repetition of
the sequence results in the structure shown below:

25    

$$R^1{-}CH{-}CH_2{-}N^+{-}N^-\left[C{-}CH_2{-}CH{-}CH_2{-}N^+{-}N^-\right]_{n-1}CO{-}CH_2{-}CH{=}CH_2$$
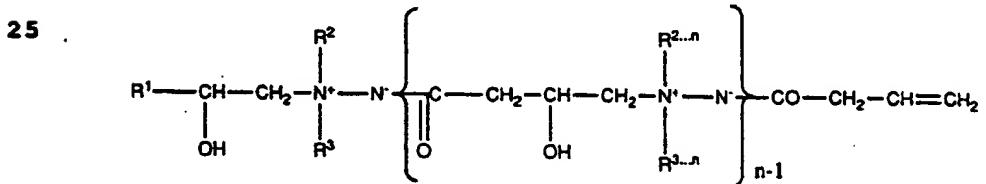
30   where the designations $R^2$ and $R^3$ are used to illustrate
the manner in which the hydrazine substituents $R^2$ and $R^3$
can be varied in each polymerization step to produce
oligomers or polymers of diverse structures.

35

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A related aminimide polymerization sequence utilizes an ester moiety bonded directly to the epoxide group.

An additional related polymerization sequence involves the use of bifunctional epoxides and esters of
5   the following form

$$H_2C\text{---}HC\text{---}(Y)\text{---}CH\text{---}CH_2 \qquad \text{and} \qquad R\text{---}O\text{---}\overset{\displaystyle O}{\underset{}{C}}\text{---}(X)\text{---}\overset{\displaystyle }{\underset{\displaystyle O}{C}}\text{---}O\text{---}R$$
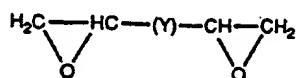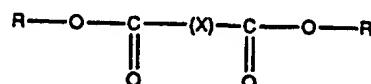
10

so as to produce polymers of the following structure (shown for the case of reaction with dimethyl hydrazine):

15

$$\left\{ \overset{\displaystyle O}{\underset{}{C}}\text{---}(X)\text{---}\overset{\displaystyle O}{\underset{}{C}}\text{---}N^-\text{---}\overset{CH_3}{\underset{CH_3}{N^+}}\text{---}CH_2\text{---}\overset{OH}{CH}\text{---}(Y)\text{---}\overset{OH}{CH}\text{---}CH_2\text{---}\overset{CH_3}{\underset{CH_3}{N^+}}\text{---}N^- \right\}$$
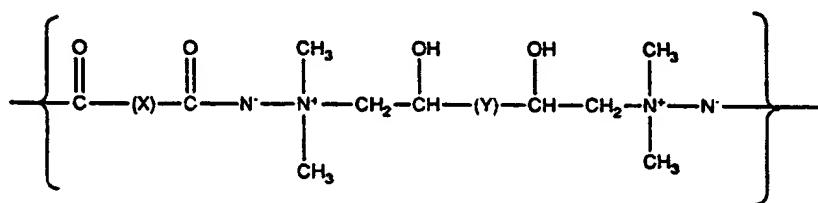
20   where X and Y are alkyl, cycloalkyl, aryl, aralkyl or alkaryl linkers.


4.2.4      Synthesis of Enantiomerically-Pure Aminimides

Enantiomerically-pure aminimides may be
25   produced by acylation of chiral hydrazinium salts as shown in the example below.

30

$$\text{HO CH}_2\text{CH}_2 \diagdown \underset{H_2N \diagup \quad \diagdown CH_3}{N^+} \diagup \bigcirc \qquad I^- \qquad \xrightarrow{C_2H_5CO_2CH_3} \qquad \text{HO CH}_2\text{CH}_2 \diagdown \underset{\underset{C_2H_5}{\overset{O}{\parallel}}{C}\text{---}N^- \diagup \quad \diagdown CH_3}{N^+} \diagup \bigcirc$$

35
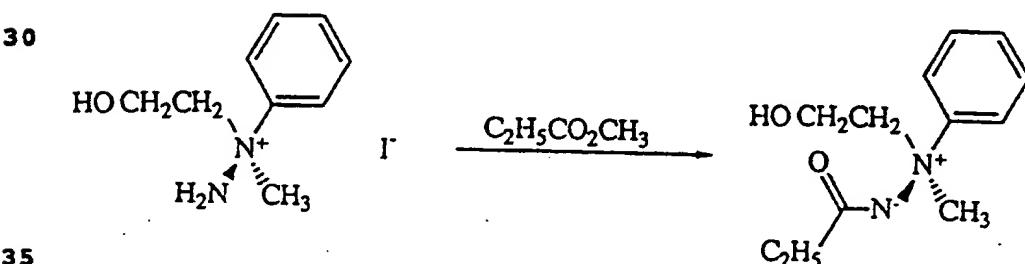
Chirally-pure hydrazinium salts may be obtained
by resolution of the racemates; resolution can be
effected by forming salts with optically pure acids, e.g.
tartaric acid, and separating the resulting diastereomers
by means of chromatography or fractional crystallization
5    (see, e.g., 103 <u>J. Chem. Soc.</u> 604 (1913)); alternatively
the racemic modification is resolved by subjecting it to
chromatographic separation using a chiral stationary
chromatographic support, or if feasible, by the use of a
suitable enzyme system.
10        Alternatively, enantiomerically-pure aminimides
may be obtained by resolution of the racemic
modifications using one of the techniques described above
for the resolution of racemic hydrazinium salts (for an
example, see 28 <u>J. Org. Chem.</u> 2376 (1963)).
15        An additional approach to the synthesis of
chiral aminimides involves chiral synthesis; an example
is provided by the reaction of (S)-(-)-propylene oxide
with 1,1-dimethylhydrazine and methyl-(R)-3-hydroxy-
butyrate, all of which are commercially available.
20
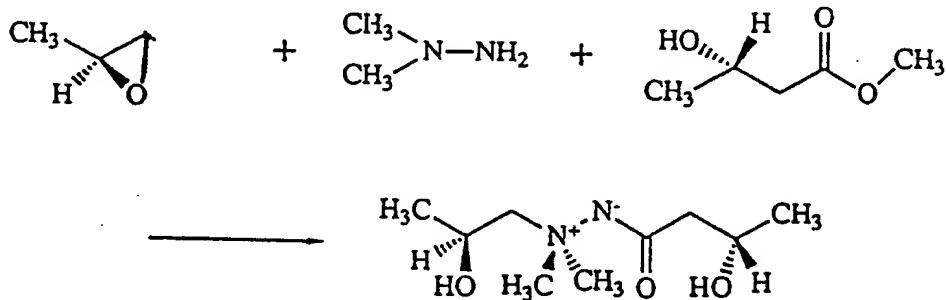


30

A variety of chiral epoxides, produced by
chiral epoxidations such as those developed by Sharpless
(Asynn. Syn., J.D. Morrison ed., Vol. 5, Ch. 7 + 8, Acad.
Press, New York, N.Y., 1985), and chiral esters, produced
35

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by standard procedures, may be used to produce a wide variety of chiral aminimides.

Chirally-pure aminimide molecular building blocks are especially preferred since they will be used to produce a vast array of molecules useful as new
5    materials for high technological applications and as molecular recognition agents, including biological ligand mimetics to be used as drugs, diagnostics, and separation agents.

10  4.4   Synthesis of Specific Classes of Aminimides

4.4.1      Synthesis of Chiral Aminimide-Containing Conjugates

The synthetic routes outlined above may be utilized to produce a wide variety of chiral aminimide
15  conjugates of the following general structure:

$$A—X—\overset{\displaystyle O}{\overset{\displaystyle \|}{C}}—N^{\cdot}-\overset{\displaystyle R^1}{\overset{\displaystyle \downarrow}{\underset{\displaystyle \overset{\vdots}{R^2}}{N^+}}}-Y—B$$
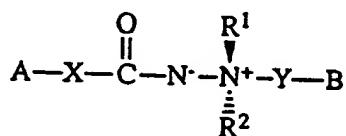
20

The substituents A and B shown may be of a variety of structures and may differ markedly in their physical or functional properties, or may be the same; they may also be chiral or symmetric.  A and B are
25  preferably selected from

1)   an amino acid derivative of the form $(AA)_n$, which would include natural and synthetic amino acid residues (n=1), peptides (n=2-30), polypeptides (n=31-70) and proteins (n>70).

30       2)   a nucleotide derivative of the form $(NUCL)_n$, which would include natural and synthetic nucleotides (n=1), nucleotide probes (n=2-25) and oligonucleotides (n>25) including both deoxyribose (DNA) and ribose (RNA)
35   variants.

3)     a carbohydrate derivative of the form $(CH)_n$. This would include natural physiologically active carbohydrates (glucose, galactose, etc.) including related compounds such as sialic acids, etc. (n=1), synthetic carbohydrate residues and derivatives of these (n=1) and all of the complex oligomeric permutations of these as found in nature (n>1) cf. Scientific American, January 1993, p. 82.

4)     a naturally occurring or synthetic organic structural motif. This term includes any of the well known base structures of pharmaceutical compounds including pharmacophores or metabolites thereof. These structural motifs are generally known to have specific desirable binding properties to ligand acceptors of interest and would include structures other than those recited above in 1), 2) and 3).

5)     a reporter element such as a natural or synthetic dye or a residue capable of photographic amplification which possesses reactive groups which may be synthetically incorporated into the oxazolone structure or reaction scheme and may be attached through the groups without adversely interfering with the reporting functionality of the group. Preferred reactive groups are amino, thio, hydroxy, carboxylic acid, acid chloride, isocyanate alkyl halides, aryl halides and oxirane groups.

6)     an organic moiety containing a polymerizable group such as a double bond or other functionalities capable of undergoing condensation polymerization or copolymerization.  Suitable groups include

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vinyl groups, oxirane groups, carboxylic acids,
acid chlorides, esters, amides, lactones and
lactams.

    7)   a macromolecular component, such as a
macromolecular surface or structures which may
be attached to the oxazolone modules via the
various reactive groups outlined above in a
manner where the binding of the attached
species to a ligand-receptor molecule is not
adversely affected and the interactive activity
of the attached functionality is determined or
limited by the macromolecule.  The molecular
weight of these macromolecules may range from
about 1000 Daltons to as high as possible.
They may take the form of nanoparticles ($d_p$=100-
1000Å), latex particles ($d_p$=1000Å-5000Å), porous
or non-porous beads ($d_p$=0.5$\mu$-1000$\mu$), membranes,
gels, macroscopic surfaces or functionalized or
coated versions or composites of these.

Under certain circumstances, A and/or B may be a chemical
bond to a suitable organic moiety, a hydrogen atom, an
organic moiety which contains a suitable electrophilic
group, such as an aldehyde, ester, alkyl halide, ketone,
nitrile, epoxide or the like, a suitable nucleophilic
group, such as a hydroxyl, amino, carboxylate, aminde,
carbanion, urea or the like, or one of the R groups
defined below.  In addition, A and B may join to form a
ring or structure which connects to the ends of the
repeating unit of the compound defined by the preceding
formula or may be separately connected to other moeities.

    A more generalized presentation of the
composition of the invention is defined by the structure

$$A-X-\left[CO-N^{-}-N^{+}-G^{1...n}\left(\begin{array}{c}R^{1...n}\\|\\|\\R^{'1...n}\end{array}\right)\right]_{n}-Y-B$$
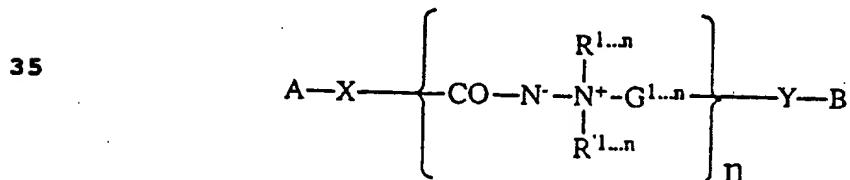
wherein:

  a.   at least one of A and B are as
defined above and A and B are optionally
connected to each other or to other compounds;

  b.   X and Y are the same or different and
each represents a chemical bond or one or more
atoms of carbon, nitrogen, sulfur, oxygen or
combinations thereof;

  c.   R and R' are the same or different
and each is an alkyl, cycloalkyl, aryl, aralkyl
or alkaryl group or a substituted or
heterocyclic derivative thereof, wherein R and
R' may be different in adjacent n units and
have a selected stereochemical arrangement
about the carbon atom to which they are
attached;

  d.   G is a chemical bond or a connecting
group that includes a terminal carbon atom for
attachment to the quaternary nitrogen and G may
be different in adjacent n units; and

  e.   $n \geq 1$.
Preferably, if G is a chemical bond, Y includes a
terminal carbon atom for attachment to the quaternary
nitrogen; and if n is 1 and X and Y are chemical bonds, R
and R' are the same, A and B are different and one is
other than H or R.

  In one embodiment of the invention, at least
one of A and B represent an organic or inorganic
macromolecular surface.  Examples of preferred
macromolecular surfaces include ceramics such as silica
and alumina, porous and nonporous beads, polymers such as
a latex in the form of beads, membranes, gels,
macroscopic surfaces or coated versions or composites or
hybrids thereof.  This functionalized surface may be
represented as follows:

$$\text{(SURFACE)}-Y-\overset{\overset{\displaystyle R^1}{|}}{\underset{\underset{\displaystyle R^2}{\vdots}}{N^+}}-N^--\overset{\overset{\displaystyle O}{\|}}{C}-X-A$$
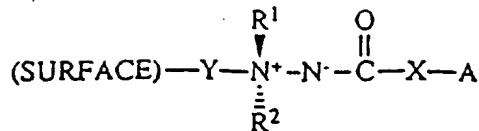
In a further embodiment of the invention, the
above roles of A and B are reversed, so that B is the
substituent selected from the foregoing list and A
represents a functionalized surface, as shown below:

$$\text{(SURFACE)}-X-\overset{\overset{\displaystyle O}{\|}}{C}-N^--\overset{\overset{\displaystyle R^1}{|}}{\underset{\underset{\displaystyle R^2}{\vdots}}{N^+}}-Y-B$$
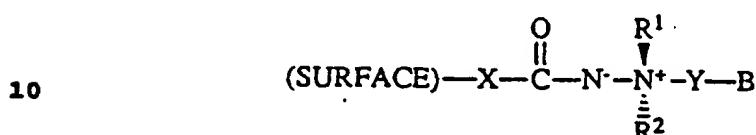
In a third preferred embodiment of the
invention, either A, B, or both contain one or more
double bonds capable of undergoing free-radical
polymerization or copolymerization to produce achiral or
chiral oligomers, polymers, copolymers, etc.

Another embodiment of the invention relates to
a composition having the structure

$$A---Y---G---\overset{\overset{\displaystyle R}{|}}{\underset{\underset{\displaystyle R^{\cdot}}{|}}{N^+}}---N---W$$
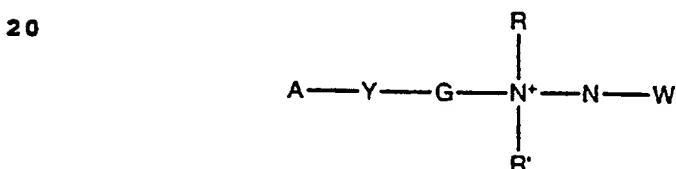
wherein A, Y, R, $R^1$ and G are as defined above and
        W is -H
or $-H_2X^-$ where $X^-$ is an anion, such as a halogen or tosyl
anion.

Yet another aspect of the invention relates to
a lipid mimetic composition having the structure
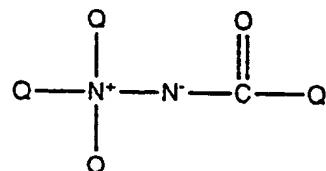
$$Q---\overset{\overset{\displaystyle Q}{|}}{\underset{\underset{\displaystyle Q}{|}}{N^+}}---N^--\overset{\overset{\displaystyle O}{\|}}{C}---Q$$

**SUBSTITUTE SHEET**

wherein Q is a chemical bond; an electrophilic
group; a nucleophilic group; R; an amino acid derivative;
a nucleotide derivative; a carbohydrate derivative; an
5   organic structural motif; a reporter element; an organic
moiety containing a polymerizable group; a macromolecular
component; or the substituent X(T) or X(T)$_2$; wherein R is
an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a
substituted or heterocyclic derivative thereof, and T is
10  a linear or branched hydrocarbon having between 12 and 20
carbon atoms some of which are optionally substituted
with oxygen, nitrogen or sulfur atoms or by an aromatic
ring; and provided that at least two T substituents are
present in the structure of the composition.
15         In the description that follows, R$^n$ where n is
an integer will be used to designate a group from the
definition of R and R$^1$.

Another aspect of the invention relates to
functionalized polymers having the structure:
20

$$(\text{SURFACE})-CH-CH_2 - \left[ \begin{array}{c} R^1 \\ | \\ N^+ - N^- - C - X - C - N - N - CH_2 \cdot CH - Y - CH - CH_2 \\ | \quad\quad \| \quad\quad \| \quad\quad | \quad\quad | \quad\quad | \\ R^1 \quad\quad O \quad\quad O \quad\quad R^2 \quad OH \quad OH \end{array} \right]_n \begin{array}{c} R^{2n+1} \\ | \\ N^+ - N^- - C - X - C - OR \\ | \quad\quad \| \quad\quad \| \\ R^{2n+1} \quad O \quad\quad O \end{array}$$

25                                     or

$$(\text{SURFACE})-C - \left[ \begin{array}{c} R^1 \\ | \\ N - N^+ - CH_2 \cdot CH - (Y) - CH \cdot CH_2 - N - N - C - (X) - C \\ | \quad\quad | \quad\quad | \quad\quad | \quad\quad \| \quad\quad \| \\ R^1 \quad OH \quad OH \quad R^2 \quad O \quad O \end{array} \right]_n - OR$$

30
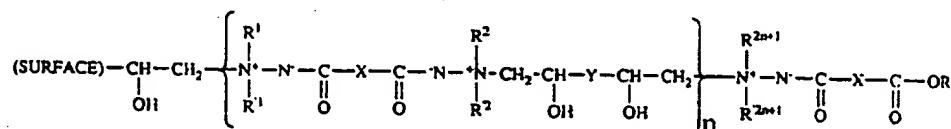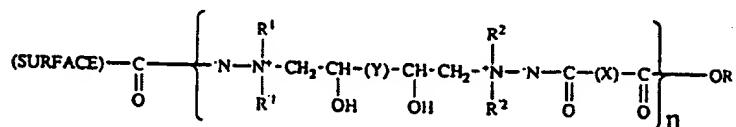
wherein

    a.   X and Y are connecting groups;
    b.   R$^n$ or R$^{'n}$ (where n = an integer) each
35         represent alkyl, cycloalkyl, aryl, aralkyl
           and alkaryl;

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    c.    (STRUCTURE) is a macromolecular component;
        and

    d.    n $\geq$ 1.

The invention also contemplates various methods of producing an aminimide-functional support.  One method comprises the steps of reacting a polymer or oligomer containing pendant moieties of OH, NH or SH with a compound of the formula:



wherein $R^1$ and $R^2$ each represent alkyl, cycloalkyl, aryl, aralkyl or alkaryl, and $R^3$ is an amino acid derivative; a nucleotide derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component; coating the reacted polymer or oligomer onto a support to form a film thereon; and heating the coated support to crosslink the film.

Another method comprises the steps of coating a mixture of multifunctional esters and multifunctional epoxides onto a support to form a film thereon; and reacting the coated support with 1,1'-dialkylhydrazine to crosslink the film.

A third method comprises the steps of coating a mixture of an aminimide-functional vinyl monomer, a difunctional vinyl monomer and a vinyl polymerization initiator onto a support to form a film thereon; and heating the coating support to form a crosslinked film.

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The aminimide-functionalized support prepared according to the previous methods are another aspect of the invention.

The ability to derivatize an aminimide scaffold in numerous ways using the synthetic techniques outlined above as well as those given below, offers a vast array of structures capable of recognizing specific molecular entities *via* establishment of specific types of binding interactions. Thus the aminimide shown below is in principle capable of establishing the following interactions: $\pi$-stacking involving the phenyl group; hydrogen bonds; acid-base interactions involving the anionic nitrogen; salt bridges involving the quarternary nitrogen; steric interactions with the bulky isopropyl substituent; and hydrophobic interactions involving the hydrocarbon chain.
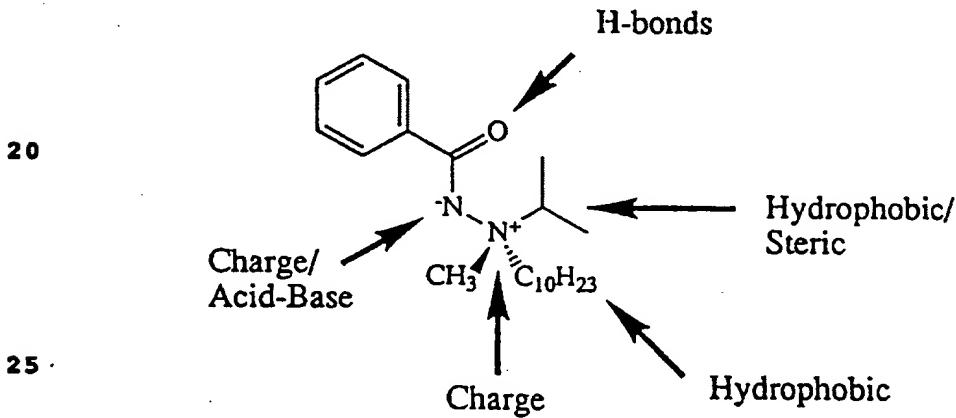


As a further example, possible interactions between a recognition target and a specific supported aminimide are shown below. Experimental procedures for the synthesis of specific chiral aminimides are given below.

Charge/
Acid-Base

H-bonds
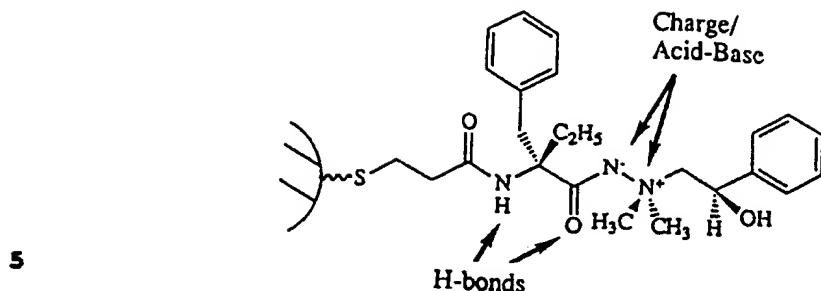
**4.4.2      Sequential Catenation of Aminimide Subunits**
             **Producing Sequences of Various Sizes**

By choosing aminimide building blocks
possessing functional groups capable of establishing
predictable binding interactions with target molecules,
and using synthetic techniques such as those broadly
described above to effect catenation (linking) of the
building blocks, it is possible to construct sequences of
aminimide subunits mimicking selected native oligomers or
polymers, e.g. peptides and polypeptides, which have
better stability and pharmacokinetic properties than
those of the native sequencers.  Specific syntheses of
multisubunit aminimides are outlined below.

**4.4.2.1     Catenation of Aminimide Subunits**
             **via Alkylation/Acylation Cycles**

The following steps are involved in this
synthesis:

1.    Acylation of a chiral hydrazinium salt,
prepared as described above, with a molecule capable of
functioning both as an acylating and as an alkylating
agent producing an aminimide; $BrCH_2COCl$ and other
bifunctional species, such as bromoalkyl isocyanates, 2-
bromoalkyl oxazolones, etc., may be used as acylating
agents under the reaction conditions given above.
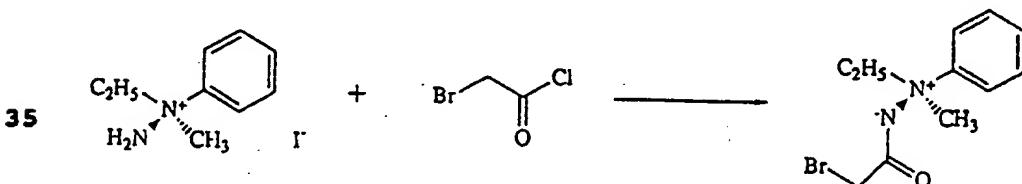
2. Reaction of the product of the above
reaction with an asymmetrically disubstituted hydrazine
to form a diastereomeric mixture of aminimide hydrazinium
salts under reaction conditions similar to those
described above.
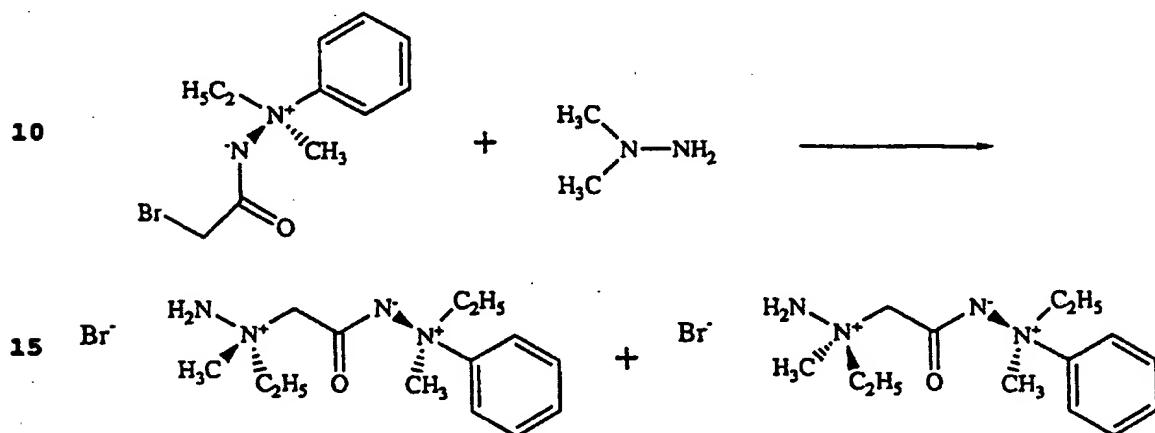


3. Isolation of the diastereomers produced in
step 2 as described above, e.g. by fractional
crystallization or by chromatography using techniques
familiar to those skilled in the art.

4. Acylation of the desired diastereomer from
step 3 with a bifunctional acyl derivative similar to
those listed in step 1 above producing a dimeric type
structure.
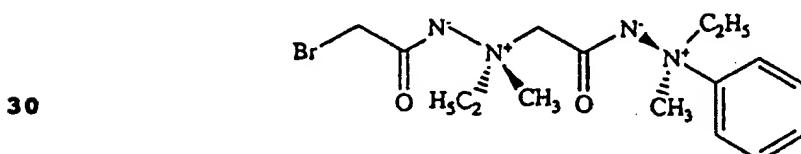


5. Repetition of steps 2, 3 and 4 the
required number of times to build the desired aminimide
subunit sequence.

6.    Capping of the assembled sequence if
desired, for example, by reaction with an acylating
agent, such as acetyl chloride.

The experimental conditions (e.g. reaction-
solvent, temperature and time, and purification
procedures for products) for all of the above reactions
were described above and are also well-known and
practiced in the art.  As the molecular weight of the
products increases (e.g. in step 5 above) solubility and
reaction-rate problems may develop if the reactions are
run under the conditions that successfully gave products
of much smaller molecular weight.  As is well known from
the art of peptide synthesis, this is probably due to
conformational (folding) effects and to aggregation
phenomena, and procedures found to work in the related
peptide cases are expected to be very useful in the case
of aminimide catenations.  For example, reaction solvents
such as DMF, or N-methyl pyrollidone, and chaotropic
(aggregate-breaking) agents, such as urea, are expected
to be helpful in alleviating reactivity problems as the
molecular-weight of the product increases.
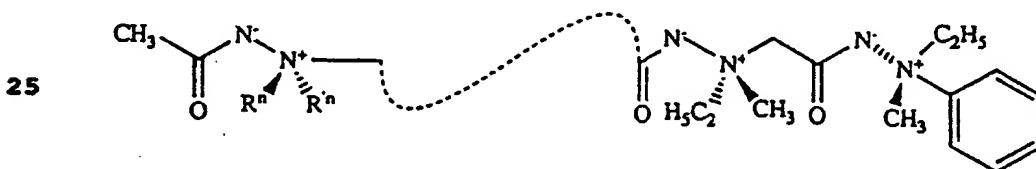


4.4.2.2   Catenation of Aminimide Subunits via
          Acylation/Alkylation Cycles

The following steps are involved in this
synthesis; experimental conditions for running the
reactions are given above.

1.  . Alkylation of an asymmetrically
disubstituted acyl hydrazide, prepared as outlined above,

- 40 -

with a molecule capable of functioning both as an
alkylating and an acylating agent to form a racemic
mixture of aminimides; as before the use of BrCH$_2$COCl is
shown below, but other bifunctional species, such as
bromoalkyl isocyanates, 2-bromoalkyl oxazolones, etc. may
also be used.

    2.   Reaction of the racemate from above with
an asymmetrically disubstituted hydrazine to form the
hydrazone:
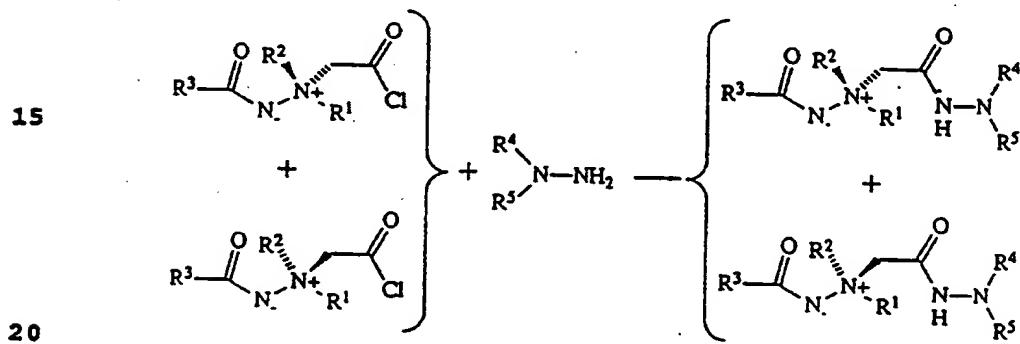


    3.   Resolution of the racemic modification
from the previous step as described above.

    4.   Alkylation of the product from step 3 with
a bifunctional molecule capable of alkylation and
acylation, which may be the same as that used in step 1
or different, to form a mixture of diastereomeric
aminimides.

    5.   Reaction of the diastereomers from step 4
with a suitable asymmetrically disubstituted hydrazine to
form the diastereomeric hydrazones, as shown:

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+
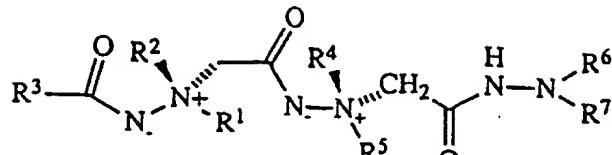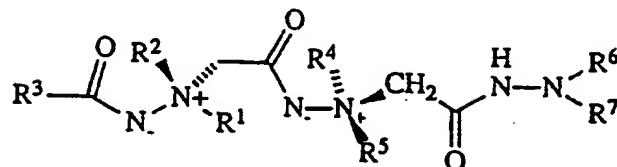


6.    Separation of the diastereomers as described above.

7.    Repetition of steps 4, 5 and 6 to build the desired sequence of aminimide subunits.

8.    Capping of the sequence, if desired, using e.g. methyl bromide to produce a sequence such as shown below.



### 4.4.2.3    Catenation of Aminimide Subunits Using Hydrazinolysis of an Ester in the Presence of an Epoxide

The following steps are involved in this synthesis; experimental conditions for running the reactions are given above.

1.    Formation of an aminimine from the reaction of an 1,1-asymmetrically disubstituted hydrazine with an epoxide; the reaction is illustrated for a chiral

epoxide below (the chiral epoxide may be obtained by e.g.
a Sharples epoxidation):
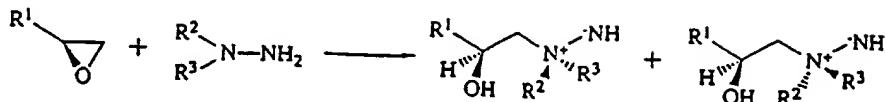


The aminimine is normally not isolated, but used directly
for the following reaction.

2. The aminimine is reacted with an ester-
epoxide to give an aminimine; for the mixture of
diastereomeric aminimides above and the ester-epoxide
shown below, the following is obtained.
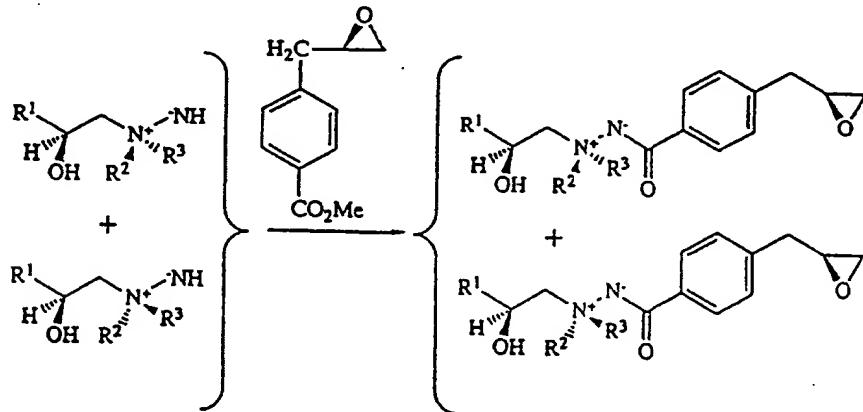


3. Separation of the diastereomeric
aminimides as described above.

4. Reaction of the desired diastereomeric
aminimide with an asymmetrically disubstituted hydrazine
to form diastereomeric aminimide-aminimines:

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+
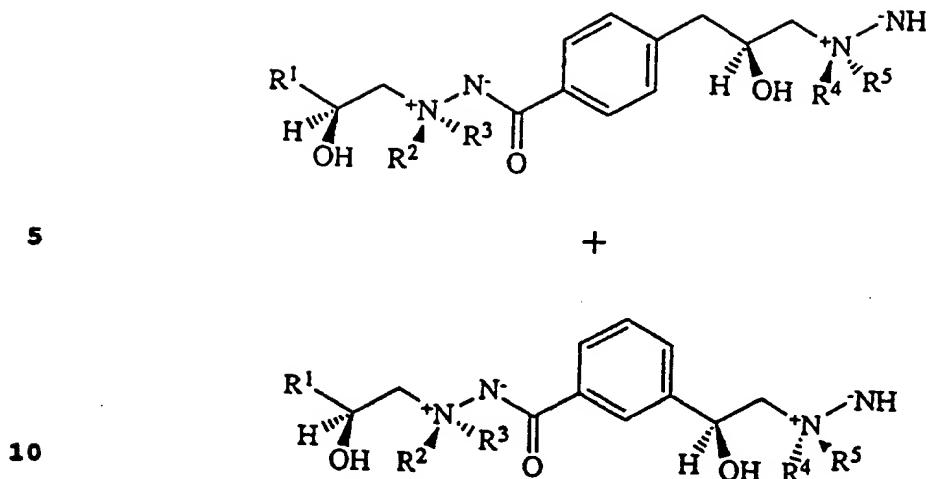


5.    Repetition of steps 2, 3 and 4 above using the appropriate hydrazines and epoxy-esters in each step to produce the desired aminimide sequence.

6.    "Capping" of the final sequence, if desired, by acylation with a simple ester, such as methyl acetate, to produce the designed aminimide ligand shown:
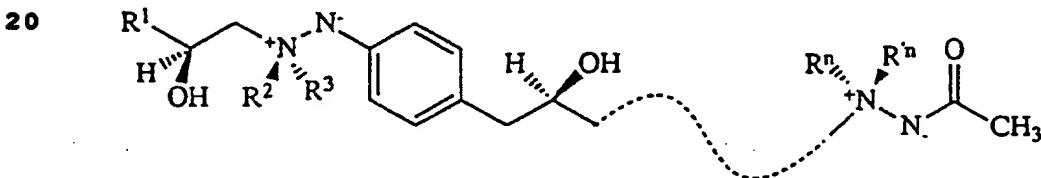


## 4.4.2.4    Catenation of α-Hydrazinium Esters or Carboxylic Acids

The following steps are involved in this synthesis; experimental conditions for running the reaction are given above.

1.    Treatment of a chirally-pure hydrazinium salt (produced as described above) with a strong base, such as NaOMe in an alcohol solvent, to form the imino anion:

$$R^1 \diagdown \overset{+}{N} \diagup NH_2 \quad X^- \quad \xrightarrow{\text{NaOMe}} \quad R^1 \diagdown \overset{+}{N} \diagup {}^-NH$$
$$R^2 \diagup {}^\backprime R^3 \qquad\qquad\qquad\qquad\qquad R^2 \diagup {}^\backprime R^3$$
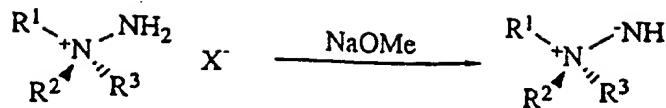
2.    Addition of an α-hydrazinium ester (again produced as discussed above) to an appropriately blocked imino-anion-containing mixture from step 1 to form the hydrazinium-aminimide, as shown.

$$R^1 \diagdown \overset{+}{N} \diagup {}^-NH \quad + \quad ROOC \diagdown \overset{+}{N} \diagup NHB_1 \quad \longrightarrow \quad R^1 \diagdown \overset{+}{N} \diagup \overset{-}{N} \diagdown\diagup \overset{+}{N} \diagup NHB_1$$
$$R^2 \diagup {}^\backprime R^3 \qquad\qquad R^4 \diagup {}^\backprime R^5 \qquad\qquad\qquad R^2 \diagup {}^\backprime R^3 \; O \;\; R^4 \diagup {}^\backprime R^5$$
$$\qquad\qquad\qquad\qquad X^- \qquad\qquad\qquad\qquad\qquad\qquad X^-$$
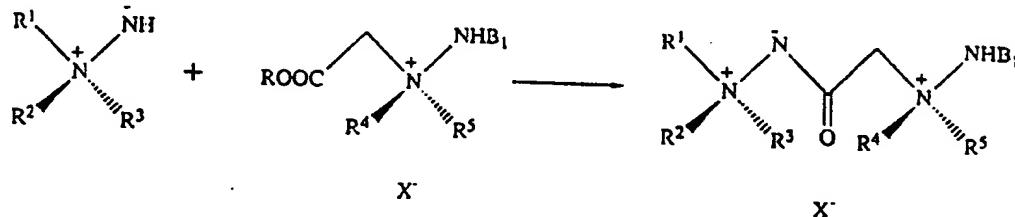
In the equation above, B1 is an appropriate protecting group such as BOC (t-butoxy carbonyl.

3.    Removal of B1 followed by repetition of steps 1 and 2 the required number of times to obtain the desired aminimide sequence, followed by a "capping" step, using a simple ester as acylating agent.

$$\left( \begin{array}{c} R^n \diagdown \diagup R^{1n} \; O \\ \overset{+}{N} \\ \overset{-}{N} \diagdown\diagup \diagdown\diagup \end{array} \right)_n$$
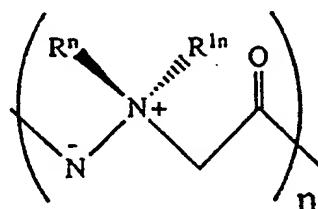
Alternatively, the α-hydrazinium carboxylic acids may be obtained by treatment of the esters with LiOH in MeOH/H₂O at room temperature, as described above, and coupled with each other using condensation reactions

promoted by DCC or other agents. Protecting groups used
in traditional peptide synthesis are expected to be
useful here as well.


4.4.3       Synthesis of Aminimide-Containing
            Peptides and Proteins

            Aminimide subunits may be introduced into any
position of a polypeptide *via* chemical synthesis, using
one of the procedures outlined above, including the
techniques for dealing with problematic reactions of high
molecular weight species. The resulting hybrid molecules
are expected to have improved properties over the native
molecules; for example, the aminimide group may confer
greater hydrolytic and enzymatic stability to the hybrid
molecule over its native counterpart.

            As an example of a synthesis of an aminimide-
modified peptide, the modification of a peptide attached
to a Merrifield solid phase synthesis support by
alkylation with aminimide-containing molecule is shown
below.
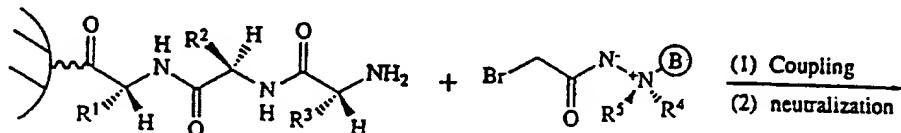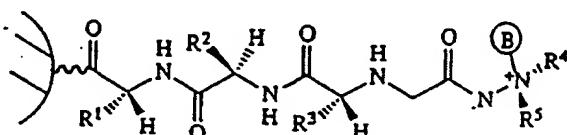


            If moiety B contains a functional group which
can be used to link additional aminimide and natural or
unnatural amino acid subunits, e.g. *via* acylation
reactions, complex hybrid structures may be obtained
using the experimental procedures outlined above.


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10
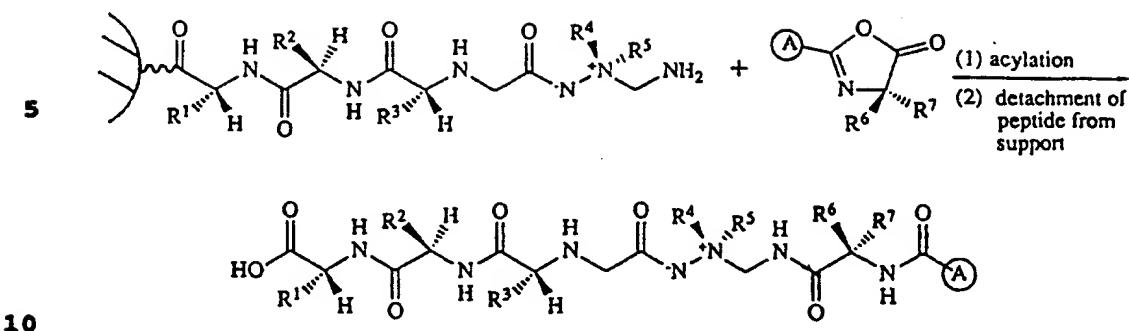
**4.4.4    Synthesis and Polymerization of Chiral**
**Aminimide-Containing Monomers**

15
      The conversion of many of the aminimide
structures described above into monomer building blocks
which can be polymerized to give novel macromolecules,
which are useful in a variety of high technological
applications, is contemplated.  The following synthetic
20
approaches are expected to be very useful in the
production of new materials.

      (a)    Free-Radical Polymerization
            of Vinyl Aminimides

      Chiral (as well as achiral) vinylaminimide
25 monomers of the general structures shown below may be
readily prepared, following the procedures outlined
above, and used in free-radical polymerizations,
according to experimental procedures well-known in the
art, to produce a vast array of novel polymeric
30 materials.
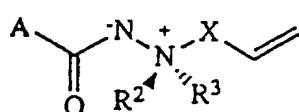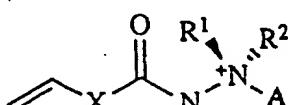
Additional monomeric structures useful in preferred free
radical polymerizations include those shown below; they
produce polymeric chains capable of being crosslinked
into more rigid structures.  The monomers shown below may
be prepared using the synthetic procedures outlined
above, and the polymerization/crosslinking reactions may
be run using standard polymerization techniques.  See,
for example, <u>Practical Macromolecular Organic Chemistry</u>,
Braun, Cherdron and Kern, trans. by K. Ivin, 3ed., Vol Z,
Harwood Academic Publishers, New York, N.Y. 1984.
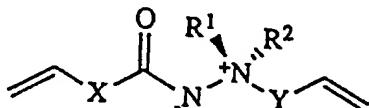


The monomers shown above may be polymerized
with other alkenes or dienes, which are either
commercially available or readily prepared using standard
synthetic reactions and techniques, to furnish copolymers
with novel structures and molecular recognition
characteristics.
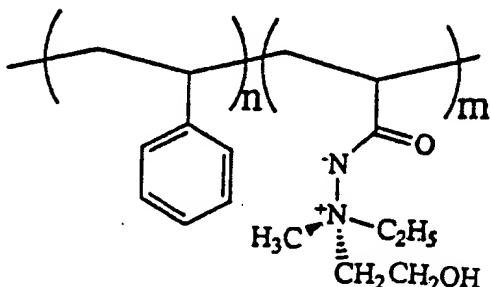
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(b)  Condensation Polymerizations Producing
     Aminimide-Containing Macromolecules

Sequential condensations of aminimide-forming
molecules may be used to produce a variety of novel
polymers of controlled size.  An example involving
5  dimeric epoxides and esters is given below; processes
involving trimeric and more complex epoxides and esters
are also contemplated; and experimental conditions for
running these polymerizations (including techniques for
resolving experimental difficulties as product molecular
10  weight increases) have been described above.
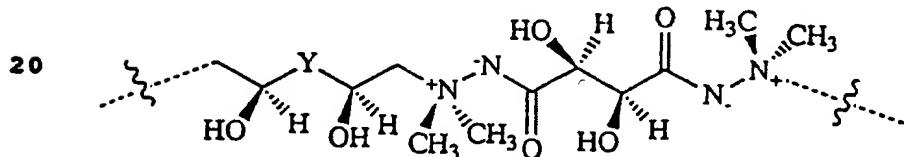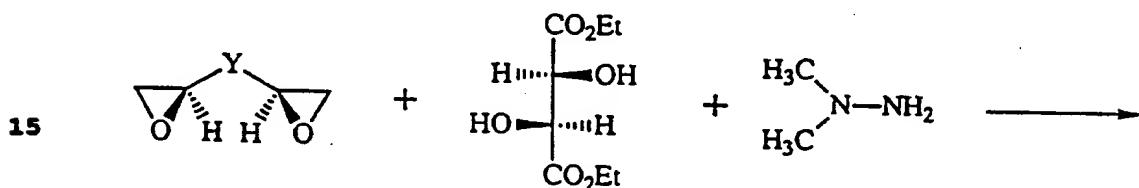


When the polymerization reaction is carried out with
25  molecules immobilized on a support, e.g. silica, a
support capable of specific molecular recognition is
produced; an example of such a support is given below:

30



35
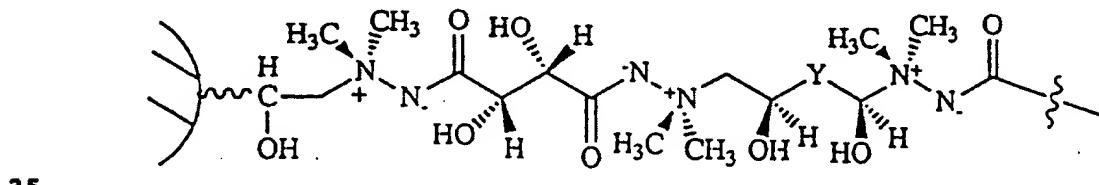
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## 4.4.5     Lipid Mimetics

Aminimide conjugate structures containing two long-chain alkyl groups capable of producing bilayer membrane structures are preferred embodiments of the present invention. Many uses of these amphiphilic, surface-active compounds are envisioned. They may be used to isolate and stabilize biologically-active molecules from the cell-wall; they are useful in the construction of affinity chromatography supports for the isolation and purification of amphiphilic macromolecules, e.g. receptors, enzymes, etc.; and they may serve as effective delivery systems for the administration of drugs.

The structure of one preferred lipid mimetic is shown below. Substituents R may be chosen from a variety of structures of various sizes including structures of ligands of biological receptors or enzymes; a preferred combination of substituents involves sterically small groups for $R_1$ and $R_2$ and a group such as A or B described above for $R_3$; the long-chain alkyl groups are 4-20 carbons in length; group X is a linker composed of atoms chosen from the set of C, H, N, O, and S.

$$
\begin{array}{c}
R_2 \\
| \\
R_1{-}\overset{+}{N}{-}\overset{-}{N}{-}\underset{\parallel}{C}{-}(X)\overset{\displaystyle chain}{\underset{\displaystyle chain}{\Big\langle}} \\
| \quad\;\; \parallel \\
R_3 \quad O
\end{array}
$$
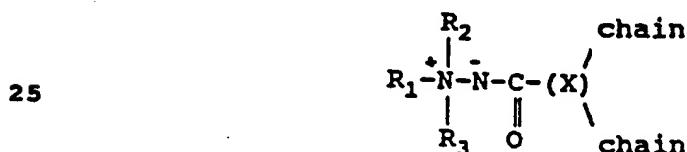
A further desirable variation of the surface-active structure shown above is as follows:

$$
\begin{array}{c}
R_2 \quad chain \\
| \\
R_1{-}\underset{\parallel}{C}{-}\overset{-}{N}{-}\overset{+}{N}{-}(X)\overset{\displaystyle chain}{\underset{\displaystyle chain}{\Big\langle}} \\
\parallel \quad\;\; | \\
O \quad\;\; R_3
\end{array}
$$

In the above structure, X is a linker group (e.g. CH);
one or more substituents R are chosen from the group of
structures A and B described above and the remaining
substituent(s) in preferably a sterically small group,
e.g. H, or CH$_3$.  An additional desirable amphiphilic
structure is shown below; substituent structures are
similar to those listed above.

$$R_1-\overset{\underset{\|}{O}}{C}-\overset{-}{N}-\overset{\overset{chain}{|}}{\overset{+}{N}}-R_2$$
$$\underset{chain}{|}$$
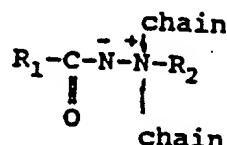
An example of a synthesis of a lipid mimetic is
given below; the required experimental conditions for the
reactions that follow are similar to those described
above for related transformations.

Thus,

$$CH_3(CH_2)_{14}-CH_2-\overset{\overset{\ominus}{\overset{/\ \backslash}{}}}{CH}-CH_2 \text{ is combined with } (CH_3)_2NNH_2 \text{ and } CH_3COOCH_3$$

to produce CH$_3$(CH$_2$)$_{14}$CH$_2$CH(OH)CH$_2$N(CH$_3$)$_2$NCOCH$_3$, which is then

combined with CH$_3$(CH$_2$)$_{14}$CH$_2$Br under alklaline conditions to

produce:

$$CH_3-(CH_2)_{15}-\overset{\underset{|}{O}}{CH}-CH_2-\overset{\overset{CH_3}{|}}{N^+}-\overset{\underset{|}{CH_3}}{N^-}-CO-CH_3$$
$$\underset{CH_3-(CH_2)_{14}-CH_2}{|}$$
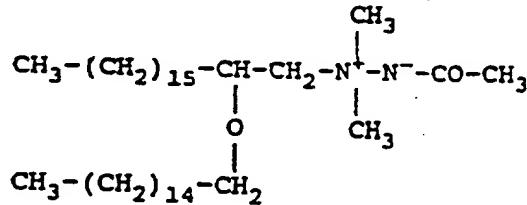
4.4.6    Fabrication of Aminimide-Containing
         Macromolecular Structures Capable of
         Specific Molecular Recognition

In an embodiment of the invention aminimide
molecular building blocks may be utilized to construct
new macromolecular structures capable of recognizing
specific molecules ("intelligent macromolecules").  The

"intelligent macromolecules" may be represented by the following general formula:

$$P - C - L - R$$

5       where,    R is a structure capable of molecular recognition;

L is a linker;

P is a macromolecular structure serving as a supporting platform;

10      C is a polymeric structure serving as a coating which surrounds P.

Structure R may be a native ligand or a biological ligand-acceptor or a mimetic thereof, such as
15 those described above.

Linker L may be a chemical bond or one of the linker structures listed above, or a sequence of subunits such as amino acids, aminimide monomers, oxazolone-derived chains of atoms, etc.

20      Polymeric coating C may be attached to the supporting platform either *via* covalent bonds or "shrink wrapping," *i.e.* the bonding that results when a surface is subjected to coating polymerization well known to those skilled in the art. This coating element may be

25      1) a thin crosslinked polymeric film 10 - 50 Å in thickness;

2) a crosslinked polymeric layer having controlled microporosity and variable thickness, or

3) a controlled microporosity gel. When the
30 support platform is a microporous particle or a membrane, as described below, the controlled microporosity gel may be engineered to completely fill the porous structure of the support platform. The polymeric coatings may be constructed in a controlled way by carefully controlling
35 a variety of reaction parameters such as the nature and

degree of coating crosslinking, polymerization initiator, solvent, concentration of reactants, and other reaction conditions, such as temperature, agitation, etc., in a manner that is well known to those skilled in the art.

5  The support platform P may be a pellicular material having a diameter (dp) from 100 Å to 1000 $\mu$, a latex particle (dp 0.1 - 0.2 $\mu$), a microporous bead (dp 1 - 1000 $\mu$), a porous membrane, a gel, a fiber, or a continuous macroscopic surface. These may be commercially available polymeric materials, such as
10  silica, polystyrene, polyacrylates, polysulfones, agarose, cellulose, etc. or synthetic aminimide-containing polymers such as those described below.

Any of the elements P, C, L, or R containing an aminimide-based structure is derived from a form of the
15  element containing a precursor to the aminimide-based structure. The multisubunit recognition agents above are expected to be very useful in the development of targeted therapeutics, drug delivery systems, adjuvants, diagnostics, chiral selectors, separation systems, and
20  tailored catalysts.

In the present specification the terms "surface", "substrate", and "structure" refer to either P, P linked to C or P linked to C and L as defined above.

Thus, another aspect of the invention relates
25  to a three-dimensional crosslinked random copolymer containing, in copolymerized form about 1 to 99 parts of a free-radically polymerizable monomer containing an aminimide group; up to 98 parts of a free-radically addition-polymerizable comonomer; and about 1 to 50 parts
30  of at least one crosslinking monomer.

The comonomer used in this copolymer may be water-soluble or water-insoluble, and the copolymer is fashioned into a water-insoluble bead, a water-insoluble membrane or a latex particle, or can be a swollen aqueous
35  gel suitable for use as an electrophoresis gel.

- 53 -

This copolymer is preferably the reaction product of about 1 to 99 parts of a condensation-polymerizable monomer containing a moiety cluster selected from the group consisting of (1) at least three epoxy groups, (2) at least three ester groups, (3) at

5   least one epoxy and at least two ester groups and (4) at least one ester and at least two epoxy groups; about 1 to 99 parts of a second condensation-polymerizable monomer containing a moiety cluster selected from the group consisting of (1) at least two ester groups, (2) at least

10  two epoxy groups and (3) at least one ester and one epoxy group; and an amount of 1,1-dialkylhydrazine equivalent, on a molar basis, substantially equal to the total molar content of epoxy groups.

15  4.4.6.1   <u>Amminimide Containing Support Materials</u>
Commercially available or readily obtainable chromatographic support materials for chromatographic and other applications, as well as other fabricated materials may be derivatized with tailored aminimide moieties,

20  through chemical modification, producing novel materials capable of recognizing specific molecular structures.
The following general structures are contemplated.

25

$$A^- (X) - \underset{\underset{O}{\overset{\|}{}}}{C} - N^- - \underset{\underset{R^2}{|}}{\overset{\overset{R^1}{|}}{N^+}} - (Y) - (SURFACE)$$

and

30

$$A - (X) - \underset{\underset{R^2}{|}}{\overset{\overset{R^1}{|}}{N^+}} - N^- - \underset{\underset{O}{\overset{\|}{}}}{C} - (Y) - (SURFACE)$$

35
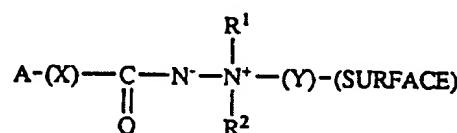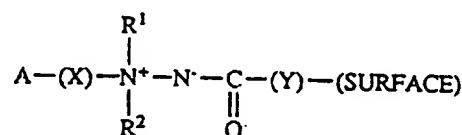
In the structures above, A is s lected from the group
consisting of amino acids, oligopeptides, polypeptides
and proteins, nucleotides, oligonucleotides,
polynucleotides, carbohydrates, molecular structures
associated with therapeutic agents, metabolites, dyes,
photographically active chemicals, and organic structures
having desired steric, charge, hydrogen-bonding or
hydrophobicity elements; X and Y are chemical bonds or
groups consisting of atoms selected from the set of C, H,
N, O, S; $R^1$ and $R^2$ are chosen from the group of alkyl,
cycloalkyl, aryl, aralkyl, alkaryl and, preferably,
structures mimicking the side-chains of naturally-
occurring amino acids.

Surfaces and other structures functionalized
with multiple aminimide subunits are also preferred;
general structures are shown below.

$$A\!-\!X\!-\!\left[\!-\!\overset{\displaystyle R^{1\ldots n}}{\underset{\displaystyle O}{\overset{\|}{C}}}\!-\!N^-\!-\!\overset{\displaystyle |}{\underset{\displaystyle R'^{1\ldots n}}{N^+}}\!-\!(Y)\!-\!\right]_n\!-\!(\text{SURFACE})$$

and

$$A\!-\!X\!-\!\left[\!-\!\overset{\displaystyle R^{1\ldots n}}{\underset{\displaystyle R'^{1\ldots n}}{N^+}}\!-\!N^-\!-\!\overset{\|}{\underset{\displaystyle O}{C}}\!-\!(Y)\!-\!\right]_n\!-\!(\text{SURFACE})$$
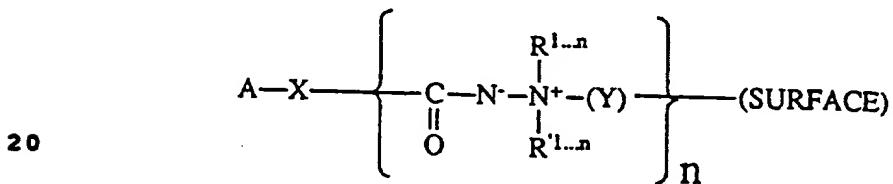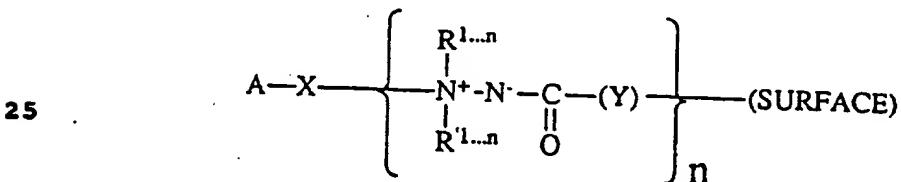
In the above structures $R^{1\ldots n}$ and $R'^{1\ldots n}$ are used to
illustrate the manner in which the hydrazine substituents
$R^1$ and $R^2$ can be varied in each polymerization step
described above to produce a functional supported
oligomer or polymer.

The following chemical modifications can be
used to prepare aminimide-functionalized surfaces.

#### 4.4.6.1.1 Functionalization of Ester and Epoxy Surfaces

A surface bearing ester groups can be treated with an epoxide, containing desired group B, and a disubstituted hydrazine to form an aminimide surface as follows:
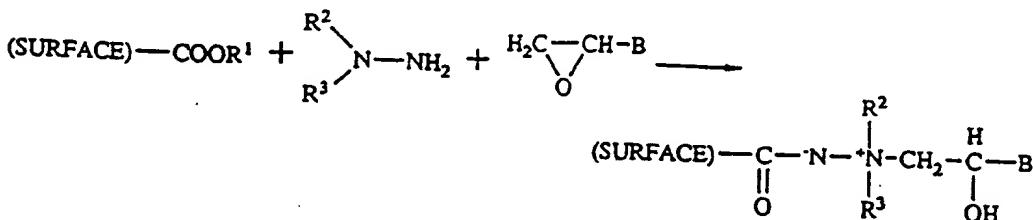
$$(SURFACE)-COOR^1 + \underset{R^3}{\overset{R^2}{>}}N-NH_2 + H_2C\overset{O}{\underset{}{\diagdown\!/}}CH-B \longrightarrow$$

$$(SURFACE)-\underset{O}{\overset{}{\underset{\|}{C}}}-N-\overset{R^2}{\underset{R^3}{\overset{|}{N}}}-CH_2-\overset{H}{\underset{OH}{\overset{|}{C}}}-B$$

To carry out the above reaction, the surface is treated with a solution containing a 10% molar excess of the epoxide (based on the calculated number of reactive ester groups of the surface), and a stoichiometric amount of the hydrazine (with respect to the amount of the epoxide) in an appropriate solvent, such as an alcohol, with shaking. The mixture is then allowed to stand at room temperature for 1 week with occasional shaking. At the end of this period, the solvent is removed by decantation, and the surface is thoroughly washed with fresh solvent and air dried.

This approach allows the functionalization of readily available supports containing ester groups.

The above reaction sequence can also be employed with an epoxide-functionalized surface:

$$(SURFACE)-\overset{H}{\underset{O}{\overset{|}{C}}}\overset{}{\underset{\diagdown\!/}{}}CH_2 + \underset{R^3}{\overset{R^2}{>}}N-NH_2 + B-COOR^1 \longrightarrow$$

$$(SURFACE)-\overset{H}{\underset{OH}{\overset{|}{C}}}-CH_2-\overset{}{\underset{R^3}{\overset{|}{N^+}}}-\overset{R^2}{\underset{}{\overset{|}{N}}}-\overset{}{\underset{O}{\overset{|}{C}}}-B$$
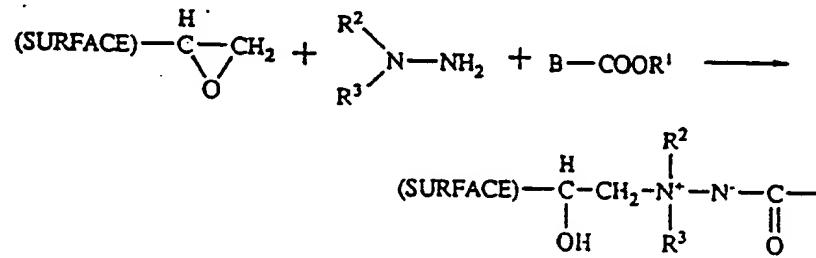
To carry out the above reaction, the surface is treated with a solution containing a 10% molar excess of the ester (based on the calculated number of reactive epoxide groups of the support), and a stoichiometric

amount of the hydrazine (with respect to the amount of
the ester used), in an appropriate solvent, such as an
alcohol, with shaking.  The mixture is then allowed to
stand at room temperature for 1 week with occasional
shaking.  At the end of this period, the solvent is
5   removed by decantation, and the surface is thoroughly
washed with fresh solvent and air dried.

The foregoing reaction can be modified by
utilizing an ester whose substituent B contains a double
bond.  After completion of the reaction shown above, the
10  double bond of the ester can be epoxidized using one of a
variety of reactions including the asymetric epoxidation
of Sharples (e.g., utilizing a peracid under suitable
reaction conditions well-known in the art), and the
product used as the epoxide in a new repetition of the
15  aminimide-forming reaction.  The overall process can be
repeated to form oligomers and polymers.

For example, using $\beta,\gamma$-butenoic acid methyl
ester as the ester, n repetitions of the above reaction
sequence produces a compound of the form:
20

$$\text{(SURFACE)}-CH-CH_2 \left[ \begin{array}{c} R^{1\ldots n} \\ | \\ -N^+-N^--C-CH_2-CH-CH_2 \\ | \quad\quad || \quad\quad | \\ R^{1\ldots n} \quad O \quad\quad OH \end{array} \right]_n -N^+-N^--C-CH_2-CH=CH_2$$
$$\quad\quad\quad\quad | \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad | \quad\quad ||$$
$$\quad\quad\quad OH \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad R^{n+1} \quad O$$
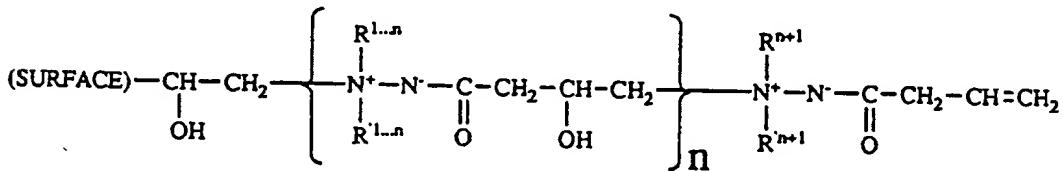
25  where the designations $R^{1\ldots n}$ and $R^{'1\ldots n}$ are used to illustrate
the manner in which the hydrazine substituents $R^2$ and $R^3$
are varied in each polymerization step, if desired, to
produce an oligomer or polymer.

The foregoing reactions can be carried out
30  using bifunctional esters of the form ROOC-X-COOR', where
X is a linker and R and R' are  alkyl groups as defined
above, and/or bifunctional epoxides of the form shown
below,

35

$$H_2C\!-\!CH\!-\!Y\!-\!CH\!-\!CH_2$$
$$\underset{O}{\diagdown\!\diagup}\qquad\qquad\underset{O}{\diagdown\!\diagup}$$
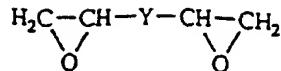
wherein Y is a linker as defined above, to form desirable polymers. If an ester-functionalized surface is reacted with bifunctional esters and epoxides, the resulting surface will have the following general structure.

$$\text{(SURFACE)}\!-\!\underset{\underset{O}{\parallel}}{C}\!-\!\left[\!-N\!-\!\underset{\underset{R^1}{|}}{\overset{\overset{R^1}{|}}{N^+}}\!-CH_2\!-CH\!-\!(Y)\!-\underset{\underset{OH}{|}}{\overset{}{CH}}\!-CH_2\!-\!\overset{+}{N}\!-N\!-\!\underset{\underset{O}{\parallel}}{C}\!-\!(X)\!-\!\underset{\underset{O}{\parallel}}{C}\!\right]_{\!n}\!\!-OR$$
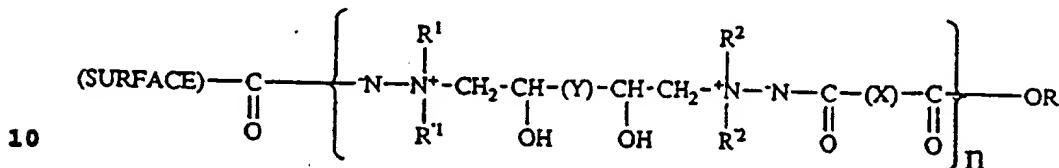
If an epoxide-functionalized surface is reacted as above the derivatized surface will have the following general structure.

$$\text{(SURFACE)}\!-\!\underset{\underset{OH}{|}}{CH}\!-\!CH_2\!-\!\left[\!\overset{\overset{R^1}{|}}{\underset{\underset{R^1}{|}}{N^+}}\!-N'\!-\!\underset{\underset{O}{\parallel}}{C}\!-X\!-\!\underset{\underset{O}{\parallel}}{C}\!-N\!-\!\overset{\overset{R^2}{|}}{\underset{\underset{R^2}{|}}{N^+}}\!-CH_2\!-CH\!-Y\!-\underset{\underset{OH}{|}}{CH}\!-CH_2\!\right]_{\!n}\!\!\overset{\overset{R^{2n+1}}{|}}{\underset{\underset{R^{2n+1}}{|}}{N^+}}\!-N'\!-\!\underset{\underset{O}{\parallel}}{C}\!-X\!-\!\underset{\underset{O}{\parallel}}{C}\!-OR$$
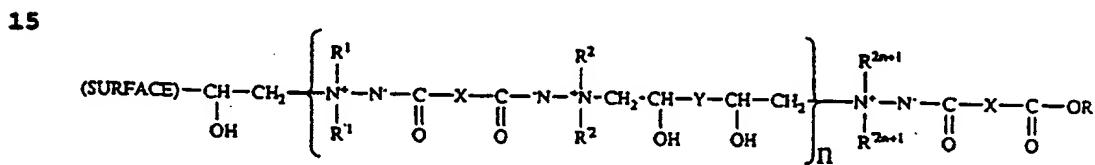
4.4.6.1.2  Functionalization of Amine Surfaces

An amine-functionalized surface can be converted to an ester-bearing surface by reaction with an acrylic ester as shown in sequence (a) below. This reaction is followed by reaction with hydrazine and an epoxide as shown in sequence (b).

$$\text{(SURFACE)}\!-\!NH_2 + H_2C\!=\!CH\!-\!\underset{\underset{O}{\overset{O}{\parallel}}}{C}\!-\!O\!-\!CH_3 \xrightarrow{\text{(a)}} \text{(SURFACE)}\!-\!NH\!\cdot\!CH_2\!-\!CH_2\!-\!\underset{\underset{O}{\overset{O}{\parallel}}}{C}\!-\!O\!-\!CH_3$$

$$\text{(SURFACE)}\!-\!NH\!\cdot\!CH_2\!-\!CH_2\!-\!\underset{\underset{O}{\overset{O}{\parallel}}}{C}\!-\!N\!-\!\overset{\overset{R^1}{|}}{\underset{\underset{R^2}{|}}{N^+}}\!-CH_2\!\cdot\!\underset{\underset{OH}{|}}{CH}\!-\!B$$

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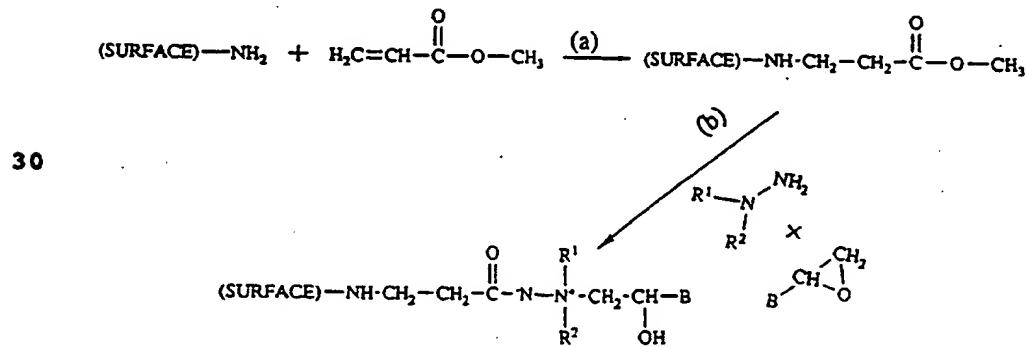
For reaction (a), a 10% molar excess of methyl acrylate (based on the number of reactive amino groups the surface as determined by a titration with acid) is dissolved in an appropriate solvent, such as an alcohol, and added to the surface. After addition is complete,

5   the mixture is shaken at room temperature for 2 days. The solvent is then removed by decantation and the surface is washed thoroughly with fresh solvent in preparation for the next step.

For reaction (b) the stoichiometric amount of a

10  1:1 mixture of the hydrazine and the epoxide, is combined in an appropriate solvent, such as an alcohol, and quickly added to the solvent-wet surface from reaction (a). The mixture is shaken at room temperature for 3 days. The solvent is then removed by decantation, and

15  the surface is washed thoroughly with fresh solvent and dried.

The above reaction sequence can also be employed with an epoxide-functionalized surface, in which case substituent B in the structure above represents the

20  surface and the desired functional group bears the amine moiety. One way of obtaining such a surface is to react a silica surface with a silicic ester containing an epoxide group to produce a so-called "epoxy silica", as shown below.

25

$$-\overset{|}{\underset{|}{Si}}-OH \ + \ (CH_3O)_3\text{-}Si\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2-CH\overset{O}{\overset{\diagup\diagdown}{-}}CH_2 \ \longrightarrow$$

30

$$-\overset{|}{\underset{|}{Si}}-O-\overset{|}{\underset{|}{Si}}\text{-}CH_2\cdot CH_2\cdot CH_2\text{-}O\text{-}CH_2\text{-}CH\overset{O}{\overset{\diagup\diagdown}{-}}CH_2$$

("Epoxy Silica")

35
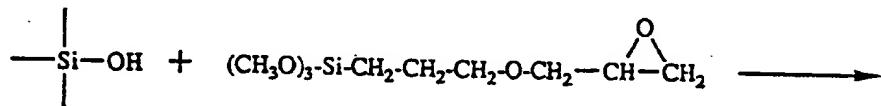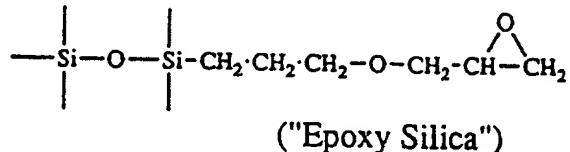
### 4.4.6.1.3 Functionalization of Carboxylic-Acid-Containing Surfaces

A surface functionalized with a carboxylic acid group can be reacted with an 1,1-dialkylhydrazine and a coupling agent, such as dicyclohexyl carbodiimide (DCC), to form a hydrazone-containing surface as shown in step (a) below. This surface can then be coupled with a desired group B bearing a substituent capable of alkylating the hydrazone to give an aminimide structure (after treatment with base), as shown in step (b):

$$\text{(SURFACE)}\!-\!\overset{\overset{\displaystyle O}{\|}}{C}\!-\!OH \;+\; H_2N\!-\!N\!\!\begin{array}{c}R^1\\R^2\end{array} \quad\xrightarrow[\text{DCC}]{\text{(a)}}\quad \text{(SURFACE)}\!-\!\overset{\overset{\displaystyle }{}}{\underset{\overset{\|}{O}}{C}}\!-\!NH\!-\!N\!\!\begin{array}{c}R^1\\R^2\end{array}$$

$$\Bigg\downarrow \overset{\text{(b)}}{\quad B\!-\!CH_2X}$$

$$\text{(SURFACE)}\!-\!\overset{\overset{\displaystyle }{}}{\underset{\overset{\|}{O}}{C}}\!-\!N\!-\!\overset{\overset{\displaystyle R^1}{|}}{\underset{\overset{|}{R^2}}{{}^+N}}\!-\!CH_2\!-\!B$$
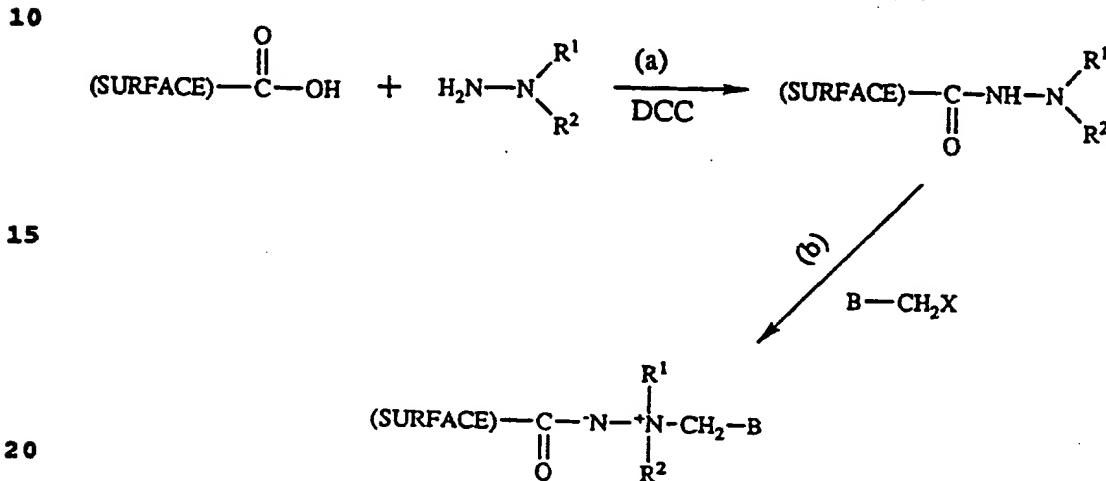
Substituent B is a surface functionalized with an alkylating agent capable of reacting with a hydrazone.

To perform the above chemical modification of a carboxyl-bearing surface, the surface is treated with a 10% molar excess equimolar amounts of the N,N-dimethylhydrazine and DCC in a suitable solvent, such as methylene chloride, and the mixture is shaken for 2 hours at room temperature. The slurry is then removed by decantation and the surface is washed thoroughly with fresh solvent to remove any residual precipitated dicyclohexyl urea. The surface is then treated with a stoichiometric amount of the alkylating agent in a suitable solvent, warmed to 70 °C and held at this temperature for 6 hours. The mixture is then cooled, the

solvent is removed by decantation, and the surface is
washed with fresh solvent and dried.

### 4.4.6.1.4 Funtionalization of Surfaces Capable of Hydrazide Alkylation

A surface bearing a group capable of alkylating
acyl hydrazones can be functionalized to contain
aminimide groups as follows:

$$\begin{array}{c} R^1 \\ \diagdown \\ \diagup \\ R^2 \end{array} N-NH-\overset{\overset{\displaystyle O}{\|}}{C}-W-B \quad + \quad (SURFACE)-Z-X \longrightarrow$$

$$(SURFACE)-Z-\overset{\overset{\displaystyle R^1}{|}}{\underset{\underset{\displaystyle R^2}{|}}{N^+}}-N^--\overset{\overset{\displaystyle O}{\|}}{C}-W-B$$
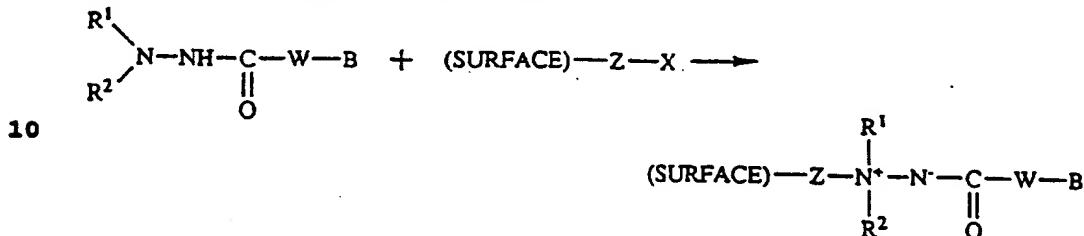
In the equation above, Z and W are linkers composed of
atoms selected from the set of C, N, H, O, S, and X is a
suitable leaving group, such as a halogen or tosylate.

A hydrazone bearing a desired group B is
produced by reacting the appropriate 1,1'-
dialkylhydrazine with any of a variety of derivatives
containing B *via* reactions that are well-known in the
art.  These derivatives may be acid halides, azlactones
(oxazolones), isocyanates,  chloroformates, or
chlorothioformates.

### 4.4.6.1.5 Functionalization of Surface Bearing -NH, -SH, or -OH Groups with Chloromethyl Aminimides

Surfaces functionalized with $-NH_2$, -SH, or -OH
groups can be functionalized by treating them with
chloromethyl aminimides in the presence of strong base
using the experimental conditions outlined above:

$$(SURFACE)-XH \quad + \quad Cl-CH_2-\overset{\overset{\displaystyle O}{\|}}{C}-N-\overset{\overset{\displaystyle R^1}{|}}{\underset{\underset{\displaystyle R^2}{|}}{N^+}}-B \longrightarrow$$

$$(SURFACE)-X-CH_2-\overset{\overset{\displaystyle O}{\|}}{C}-N-\overset{\overset{\displaystyle R^1}{|}}{\underset{\underset{\displaystyle R^2}{|}}{N^+}}-B$$

**SUBSTITUTE SHEET**
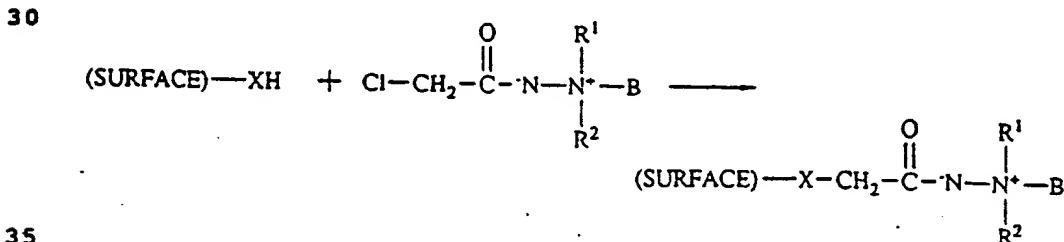
The required chloromethyl aminimides can be
prepared by known literature procedures (See, e.g., 21 J.
Polymer Sci., Polymer Chem. Ed. 1159 (1983)), or by using
the techniques described above.

5    4.4.6.1.6 Functionalization of Oxazolone-Containing
              Surfaces
              Oxazolone-containing surfaces can be
functionalized by first reacting them with 1,1'-
dialkylhydrazine as shown in step (a) below followed by
10   alkylation of the resulting hydrazone with an alkylating
agent B-CH$_2$-X as shown in step (b); reaction conditions
similar to those described above are expected to be
effective in carrying out these modifications.
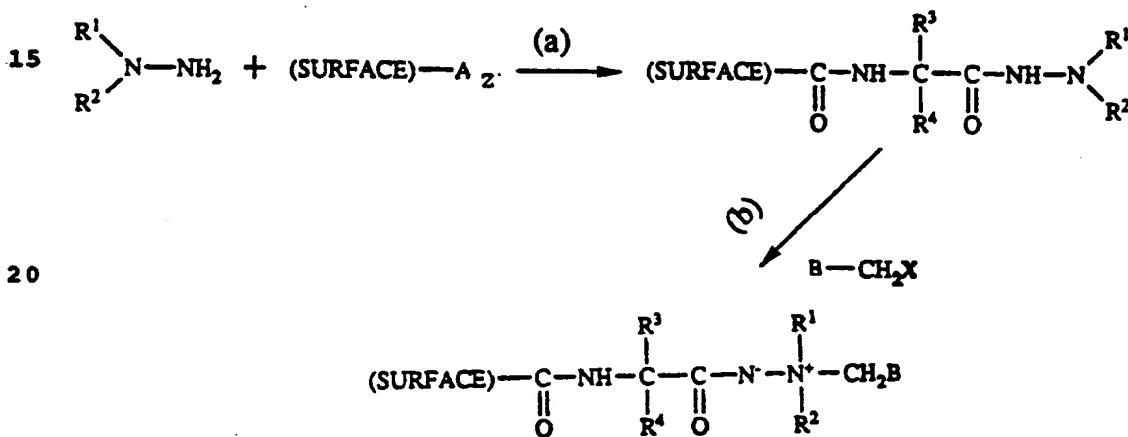


25   In the structures above, R$^3$ and R$^4$ are derived from the
five membered azlactone ring denoted by Az.
              Although the previous discussions are
specifically directed to the functionaliziation of
surfaces, these reactions can also be used to construct
30   aminamide linkages to the other speciesof A and B which
are described in this application.

     4.4.7    Preparation of Aminimide-Based
              Coatings for Support Materials
35            It is possible to produce aminimide-
functionalized composite support materials by coating

- 62 -

various solubl aminimide formulations on the surfaces of
existing supports, and subsequently crosslinking the
resulting coatings in place to form mechanically stable
surfaces.  The coating may be engineered for a particular
application (e.g., to take the form of a thin non-porous
5  film or to possess localized microporosity for enhanced
surface area) by judicious selection of process
conditions, monomer loading levels, the crosslinking
mechanism and the amount of crosslinker.

    For example, any of the foregoing reactions can
10  be carried out with a vinyl aminimide in contact with a
selected surface, which is polymerized according to well-
known techniques (see, e.g., U.S. Patent No. 4,737,560).
The polymerization results in a surface coated with a
polymer containing aminimide side-chains.  Other coating
15  procedures employing aminimide functional groups are
described below in greater detail. .

$$\text{Epoxy Silica} \;+\; \begin{array}{c} H_3C \\[-2pt] {\Large\diagdown} \\[-6pt] {\Large\diagup} \\[-2pt] H_3C \end{array}\!\!N\!-\!NH_2 \;+\; (C_2H_5)_2\!-\!N\text{-}CH_2\text{-}CH_2\!\cdot\!COOC_2H_5 \longrightarrow$$

20

$$-\overset{|}{\underset{|}{Si}}-O-\overset{|}{\underset{|}{Si}}\text{-}CH_2\!\cdot\!CH_2\!\cdot\!CH_2\text{-}O\text{-}CH_2\text{-}\underset{\underset{OH}{|}}{CH}\text{-}CH_2\text{-}\overset{\overset{CH_3}{|}}{\underset{\underset{CH_3}{|}}{N^+}}\text{-}N^-\text{-}\overset{\overset{O}{\|}}{C}\text{-}CH_2\text{-}CH_2\text{-}N(C_2H_5)_2$$
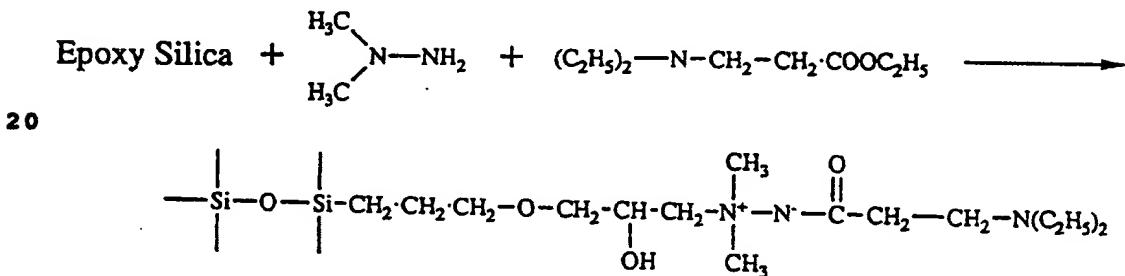
25  4.4.8      Synthesis of Aminimide-Containing Materials *Via*
            Polymerizations of Aminimide-Based Molecules
            In addition to utilizing aminimide chemistry to
chemically modify commercially available or readily
obtainable surfaces, new surfaces and other materials can
30  be fabricated *de novo* from aminimide precursors bearing
polymerizable groups by polymerizations and/or
copolymerizations in the presence or absence of
crosslinking agents.  Depending upon the properties for
the desired material, various combinations of monomers,
35  crosslinkers, and ratios thereof may be employed.  The

resultant support materials may be latex particles, porous or non-porous beads, membranes, fibers, gels, electrophoresis gels, or hybrids thereof. Furthermore, the monomers and crosslinking agents may or may not all be aminimides.

Vinyl or condensation polymerizations may be advantageously employed to prepare the desired aminimide-containing materials. Vinyl polymerization can include use of one or more monomers of the form $CH_2=CH-X$ that are copolymerizable with aminimides; suitable examples include styrene, vinyl acetate, and acrylic monomers. If desired, compatible non-aminimide crosslinkers, such as divinyl benzene, may be employed (either singly or in combination as the other such agents).

Condensation polymerization may be accomplished using multifunctional epoxides and multifunctional esters with the appropriate amounts of an 1,1'-dialkylhydrazine, using the reaction conditions described above. Either the ester component or the epoxide component should be at least trifunctional to obtain three-dimensionally crosslinked polymer structures; preferably, both components are trifunctional.

The nature and conditions of processing, the ratio of the various monomers and the ratio of crosslinker to total monomer content can be varied to produce a variety of product structures (e.g., beads, fibers, membranes, gels, or hybrids of the foregoing) and to tailor the mechanical and surface properties of the final product (e.g., particle size and shape, porosity, and surface area). Appropriate parameters for a particular application are readily selected by those skilled in the art.

4.4.9    Combinatorial Libraries of Peptidomimetics
         Derived From Aminimide Modules

The synthetic transformations of aminimides
outlined above may be readily carried out on solid
supports in a manner analogous to performing solid phase
peptide synthesis, as described by Merrifield and others
(see for example, Barany, G., Merrifield, R.B., Solid
Phase Peptide Synthesis, in The Peptides Vol. 2, Gross
E., Meienhofer, J. eds., p. 1-284, Acad. Press, New York
1980; Stewart, J.M., Yang, J.D., Solid Phase Peptide
Synthesis, 2nd ed., Pierce Chemical Co., Rockford,
Illinois 1984; Atherton, E., Sheppard, R.C., Solid Phase
Peptide Synthesis, D. Rickwood & B.D. Hames eds., IRL
Press ed. Oxford U. Press, 1989). Since the assembly of
the aminimide-derived structures is modular, i.e., the
result of serial combination of molecular subunits, huge
combinatorial libraries of aminimide-based oligomeric
structures may be readily prepared using suitable solid-
phase chemical synthesis techniques, such as those of
described by Lam (K.S. Lam, et al. Nature 354, 82 (1991))
and Zuckermann (R.N. Zuckermann, et al. Proc. Natl. Acad.
Ser. USA, 89, 4505 (1992); J.M. Kerr, et al., J. Am Chem.
Soc. 115, 2529 (1993)). Screening of these libraries of
compounds for interesting biological activities, e.g.,
binding with a receptor or interacting with enzymes, may
be carried out using a variety of approaches well known
in the art. With "solid phase" libraries (i.e.,
libraries in which the ligand-candidates remain attached
to the solid support particles used for their synthesis)
the bead-staining technique of Lam may be used. The
technique involves tagging the ligand-candidate acceptor
(e.g., an enzyme or cellular receptor of interest) with
an enzyme (e.g., alkaline phosphatase) whose activity can
give rise to color production thus staining library
support particles which contain active ligand-candidates
and leaving support particles containing inactive ligand-
candidates colorless. Stained support particles are
physically removed from the library (e.g., using tiny

.forceps that are coupled to a micromanipulator with the
aid of a microscope) and used to structurally identify
the biologically active ligand in the library after
removel of the ligand acceptor from the complex by e.*g.*,
washing with 8M guanidine hydrochloride.  With "solution-
phase" libraries, the affinity selection techniques
described by Zuckermann above may be employed.

An especially preferred type of combinatorial
library is the encoded combinatorial library, which
involves the synthesis of a unique chemical code (e.*g.*,
an oligonucleotide or peptide), that is readily
decipherable (e.*g.*, by sequencing using traditional
analytical methods), in parallel with the synthesis of
the ligand-candidates of the library.  The structure of
the code is fully descriptive of the structure of the
ligand and used to structurally characterize biologically
active ligands whose structures are difficult or
impossible to elucidate using traditional analytical
methods.  Coding schemes for construction of
combinatorial libraries have been described recently (for
example, see S. Brenner and R.A. Lerner, Proc. Natl.
Acad. Sci. USA 89, 5381 (1992); J.M. Kerr, et al. J. Am.
Chem. Soc. 115, 2529 (1993)).  These and other related
schemes are contemplated for use in constructing encoded
combinatorial libraries of oligomers and other complex
structures derived from aminimide units.

The power of combinatorial chemistry in
generating screenable libraries of chemical compounds
e.*g.*,in connection with drug discovery, has been
described in several publications, including those
mentioned above.  For example, using the "split solid
phase synthesis" approach outlined by Lam et al., the
random incorporation of 20 different aminimide units into
pentameric structures, wherein each of the five subunits
in the pentamer is derived from one  f the aminimide
units, produces a library of $20^5 = 3,200,000$

peptidomimetic ligand-candidates, each ligand-candidate is attached to one or more solid-phase synthesis support particles and each such particle contains a single ligand-candidate type. This library can be constructed and screened for biological activity in just a few days. Such is the power of combinatorial chemistry using oxazolone modules to construct new molecular candidates.

The following is one of the many methods that are being contemplated for use in constructing random combinatorial libraries of aminimides-based compounds; the random incorporation of three aminimides derived from α-chloroacetyl chloride and the hydrazines shown below to produce 27 trimeric structures linked to the support via a succinoyl linker is given below.

$$\begin{array}{ccc}
R_1 & R'_1 & R''_1 \\
| & | & | \\
N-NH_2 & N-NH_2 & N-NH_2 \\
| & | & | \\
R_2 & R'_2 & R''_2
\end{array}$$
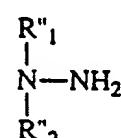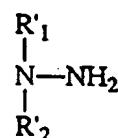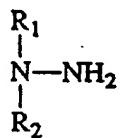
(1)  A suitable solid phase synthesis support, e.g., the chloromethyl resin of Merrifield is treated with 4-hydroxyl butyric acid in the presence of $CsCO_3$ followed by tosylation with p-toluenesulfonyl chloride, under conditions known in the art;

$$\text{Ⓟ-CH}_2\text{—Cl} \quad + \quad \text{HO-(CH}_2)_3\text{—CO}_2\text{H} \xrightarrow[\text{2. TsCl}]{\text{1. CsCO}_3}$$

$$\text{Ⓟ-CH}_2\text{-O}_2\text{C-(CH}_2)_3\text{-OTs}$$

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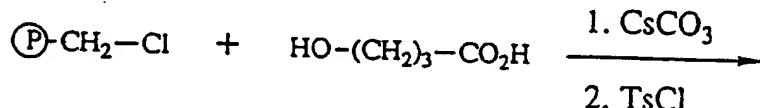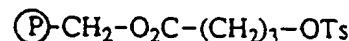
(2)  The resulting resin is split into three equal
     portions. Each portion is coupled with one of the
     hydrazines shown above to give the hydrazinium resin
     which is converted to the aminimide by reaction with
     chloroacetyl chloride using the experimental
     conditions described above.

$$\text{P}-CH_2-O_2C-(CH_2)_3-OTs \xrightarrow[\text{2. } ClCH_2COCl]{\text{1. } \underset{R}{\overset{R_1}{N}}-NH_2}$$

$$\text{P}-CH_2-O_2C-(CH_2)_3-\underset{R_2}{\overset{R_1}{N^+}}-N^--\overset{O}{\overset{\|}{C}}\diagdown Cl$$

(3)  The aminimide resin portions are mixed thoroughly
     and split again into three equal portions. Each
     resin portion is coupled with a different hydrazine
     followed by a coupling with α-chloracetyl chloride
     producing a resin with two linked aminimide
     subunits. The resin portions are then mixed
     thoroughly and split into three equal portions.

$$\text{P}-CH_2-O_2C-(CH_2)_3-\underset{R_2}{\overset{R_1}{N^+}}-N^--\overset{O}{\overset{\|}{C}}\diagdown Cl \xrightarrow[\text{2. } ClCH_2COCl]{\text{1. } \underset{R'_2}{\overset{R'_1}{N}}-NH_2}$$

$$\text{P}-CH_2-O_2C-(CH_2)_3-\underset{R_2}{\overset{R_1}{N^+}}-N^--\overset{O}{\overset{\|}{C}}-CH_2-\underset{R'_2}{\overset{R'_1}{N^+}}-N^-\diagdown\overset{O}{\overset{\|}{C}}-CH_2-Cl$$

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(4)  Each resin portion is coupled with a different
     hydrazine followed by reaction with an acid chloride
     to produce a resin with three linked aminimide
     subunits;

5
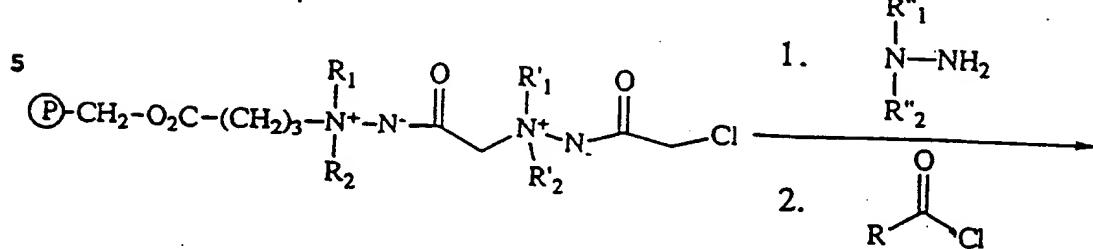


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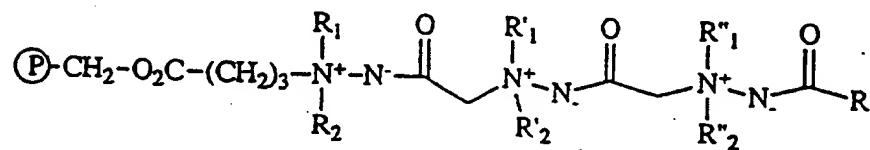


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The resin portions are mixed producing a
library containing 27 types of beads each bead type
containing a single trimeric aminimide species for
screening using the bead-stain method described above.
20  Alternatively, the aminimides may be detached from the
support via acidolysis producing a "solution-phase"
library of aminimides containing a butyrylated terminal
nitrogen.  (Shown in the structure below in which
$R=C_3H_7$)

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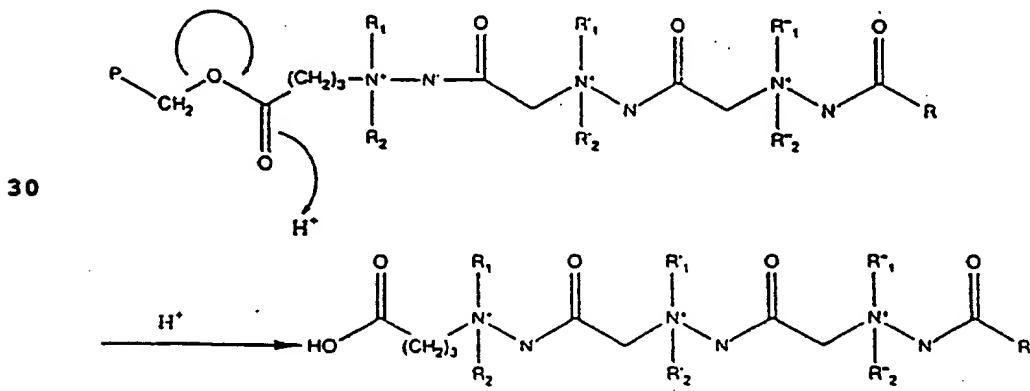
- 69 -

4.4.10    Design and Synthesis of
          Aminimide-Based Glycopeptide Mimetics

A great variety of saccharide and
polysaccharide structural motifs incorporating aminimide
structures are contemplated including, but not limited
to, the following.

(1)   Replacement of certain glycosidic linkages
by aminimide backbones using reactions well known in the
art of sugar chemistry and reactions described above.
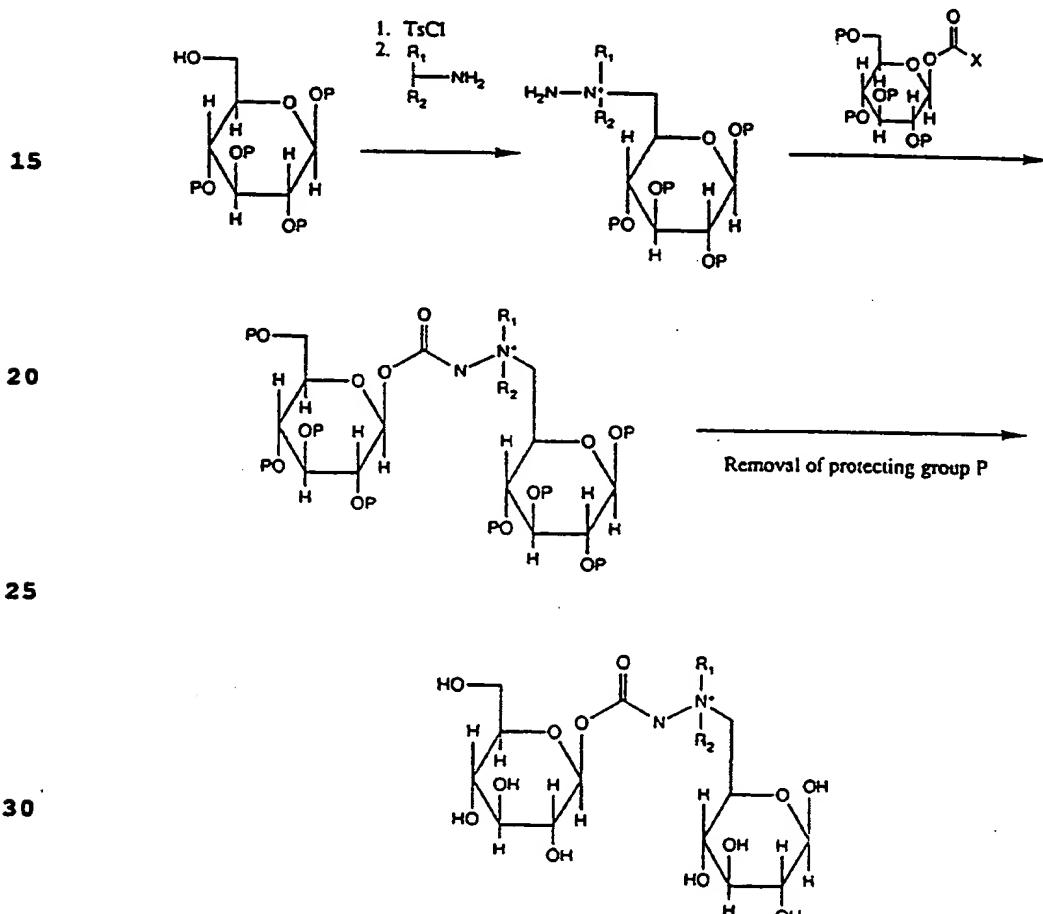
- 70 -

(2)  Use of aminimide structures as linkers
holding in place a sugar derivative and a tailored
mimetic, or another sugar.

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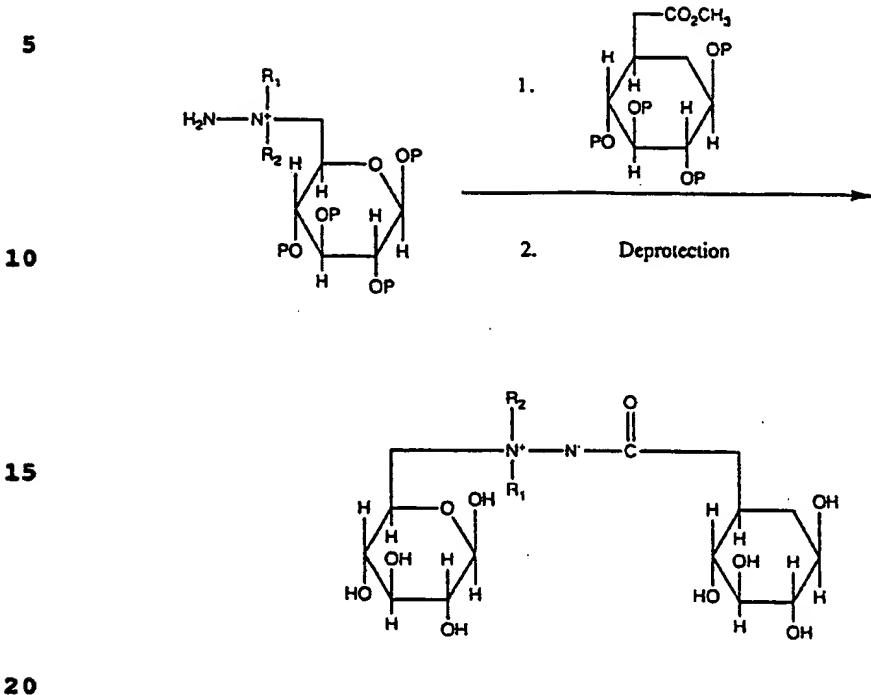
4.4.11    Design and Synthesis of
         Aminimide-Containing Oligonucleotide Mimetics

         The art of nucleotide and oligonucleotide
synthesis has provided a great variety of suitably
25  blocked and activated furanoses and other intermediates
which are expected to be very useful in the construction
of aminimide-based mimetics.  (Comprehensive Organic
Chemistry, Sir Derek Barton, Chairman of Editorial Board,
Vol. 5, E. Haslam, Editor, pp. 23-176).
30       A great variety of nucleotide and
oligonucleotide structural motifs incorporating
aminimide-based structures are contemplated including,
but not limited to, the following.
         (1)  For the synthesis of  ligonucleotides
35  containing peptidic aminimide-based linkers in place of

the phosphate diester groupings found in native oligonucleotides, the following approach is one of many that is expected to be useful.
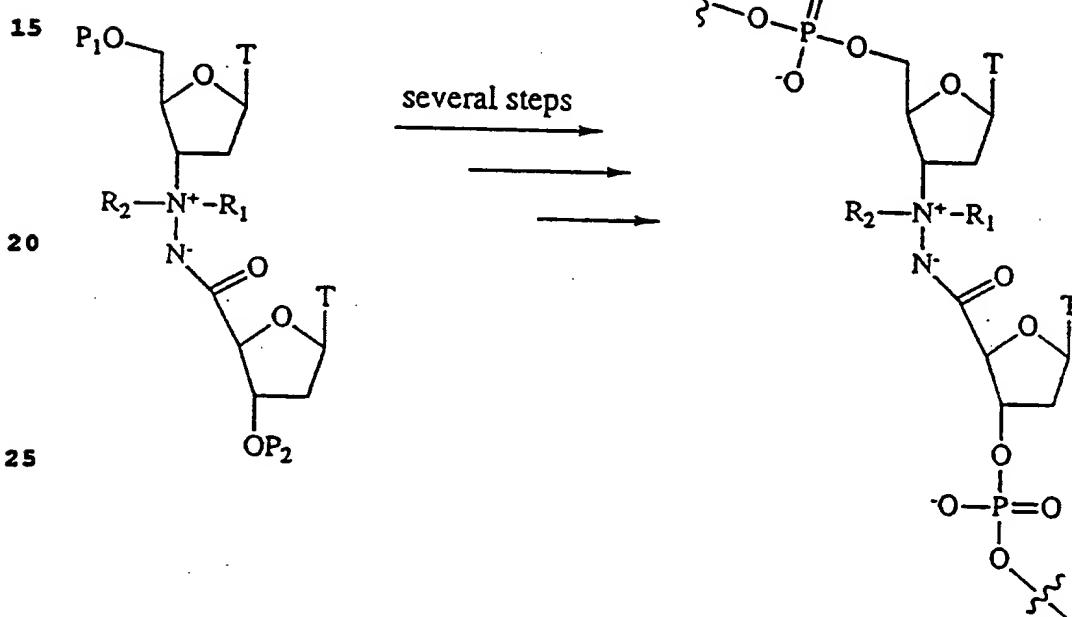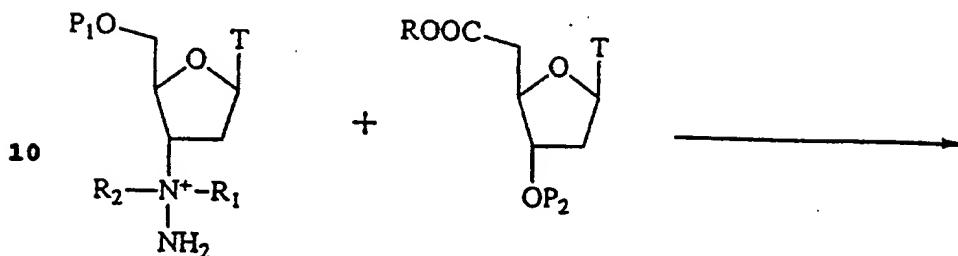
(2)  For the synthesis of structures in which an aminimide grouping is used to link complex oligonucleotide-derived units, an approach such as the following is expected to be very useful.
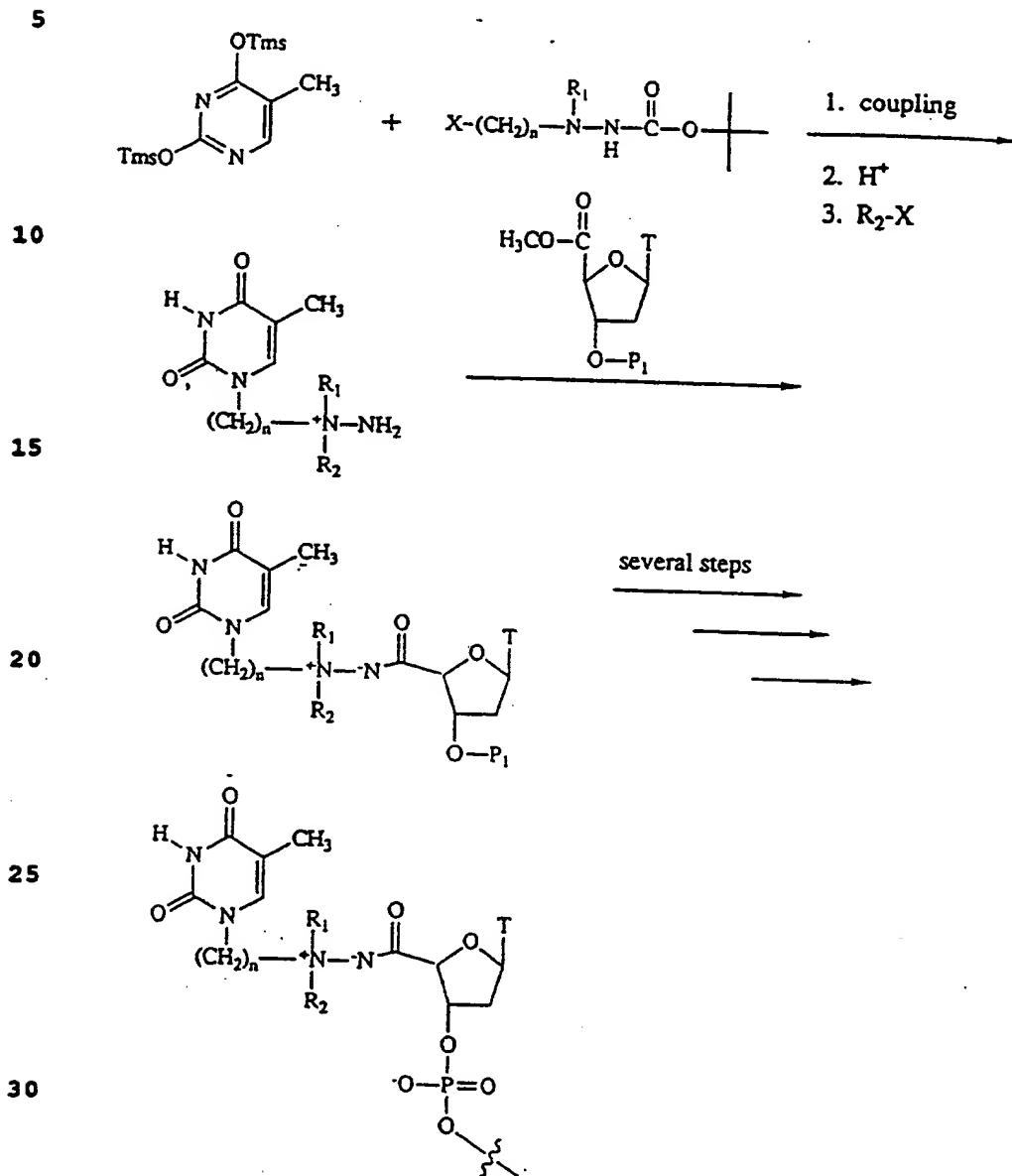
5.  EXAMPLE:  SYNTHESIS OF AN AMPHIPHILIC LIGAND
    MIMETIC USEFUL IN ISOLATION AND PURIFICATION
    OF RECEPTORS BINDING VINCAMINE

        To a solution of 1.84 g (0.01 mol) of 1,2-epoxy
dodecane (I) in a suitable solvent, such as n-propanol,

5  is added with stirring 0.61 g (0.01 mol) of 1,1-
dimethylhydrazine.  The solution is stirred for 1 hour at
room temperature, cooled to 10 °C in an ice bath, and a
solution of 3.54 g (0.01 mol) of vincamine (II) dissolved
in the minimum amount of the same solvent is added.  The

10  reaction mixture is stirred at 0 °C for 2 hours, allowed
to come to room temperature, and stirred at room
temperature for 3 days.  At the end of this time the
solvent is removed under high vacuum (0.2 torr) and the
crude product is isolated.  The conjugate (II) is useful

15  as a stabilization agent for the isolation and
purification of receptor proteins which are
therapeutically acted upon by vincamine and by
structurally related molecules.

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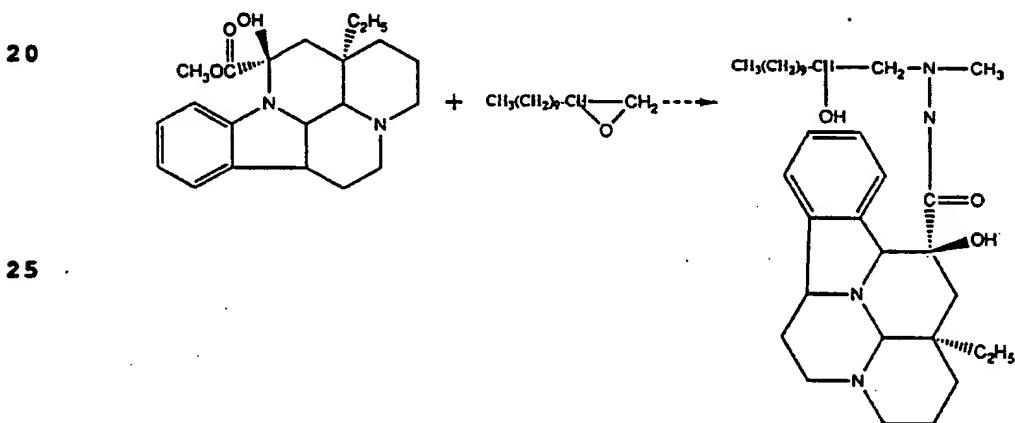
30  6.  EXAMPLE:  SYNTHESIS OF AMPHIPHILIC LIGAND
    MIMETIC USEFUL IN THE ISOLATION AND
    PURIFICATION OF SEROTONIN BINDING RECEPTOR

        8.61 g (0.1 mol) of methyl acrylate is added
over a 15 minute period to a stirred solution of 17.62 g
(0.1 mol) of serotonin in 100 ml of a suitable solvent.

35 The reaction mixture is allowed to come to room

- 74 -

temperature and stirred at room temperature for 2 days. The solvent is then removed by freeze drying to yield the ester (IV). 6.01 g (0.1 mol) of 1,1-dimethylhydrazine is added with stirring to a solution of 18.4 g (0.1 mol) of 1,2-epoxydodecane in a suitable solvent, such as

5    propanol. The mixture is stirred at room temperature for 1 hour and a solution of (IV) dissolved in the same solvent is added. The mixture is then stirred at room temperature for 3 days. At the end of this time the solvent is removed in vacuo to yield the serotonin

10   conjugate (V), which is useful as a ligand for the discovery, stabilization and isolation of serotonin-binding membrane receptor proteins.

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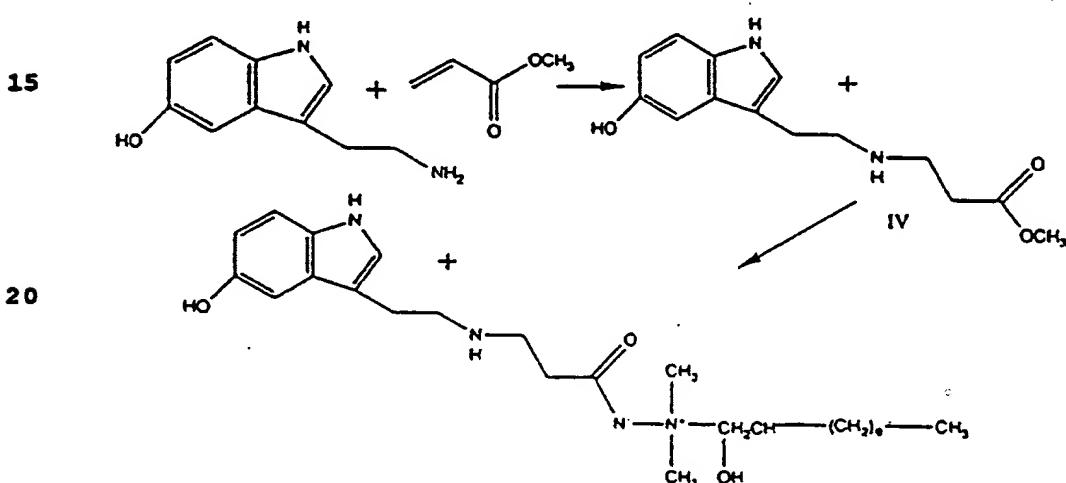
25   7.   EXAMPLE:  SYNTHESIS OF RHODAMINE-B-CONTAINING LIGAND MIMETIC USEFUL IN THE ISOLATION AND PURIFICATION OF CODEINE-BINDING PROTEINS

49.74 g (0.1 mol) of the acid chloride of Rhodamine B (VI), prepared from rhodamine B by the

30   standard techniques for preparing acid chlorides from carboxylic acids, are dissolved in 500 ml of a suitable solvent and are added, with stirring, over a 1-hour period to a solution of 6.01 g (0.1 mol) of 1,1-dimethylhydrazine in 100 ml of the same solvent. The

35   temperature is kept at 10 °C. After the addition is complete, the mixture is stirred at room temperature for

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12 hours, and the solvent is stripped away *in vacuo* to yield the Rhodamine B dimethylhydrazine (VII).

5.21 g (0.01 mol) of (VII) is dissolved in 100 ml of a suitable solvent, such as benzene, and 4.69 g (0.01 mol) of tosyl codeine (VIII), prepared from codeine
5 by the standard techniques for the tosylation of an alcohol, in 50 ml of the same solvent is added over a 15-minute period with stirring. The mixture is heated to reflux and held at reflux for 1 hour. The mixture is then cooled, the solvent is removed *in vacuo*, the residue
10 is redissolved in an appropriate alcohol and adjusted to pH 8 with 10% methanolic KOH. The precipitated salts are removed by filtration and the solvent is stripped *in vacuo* to yield the conjugate (IX), useful as a probe for the location, stabilization and isolation of receptor
15 proteins that bind codeine and structurally similar analogues.

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**IV**

**VII**

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**VII  +**

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**VIII**

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**IX**

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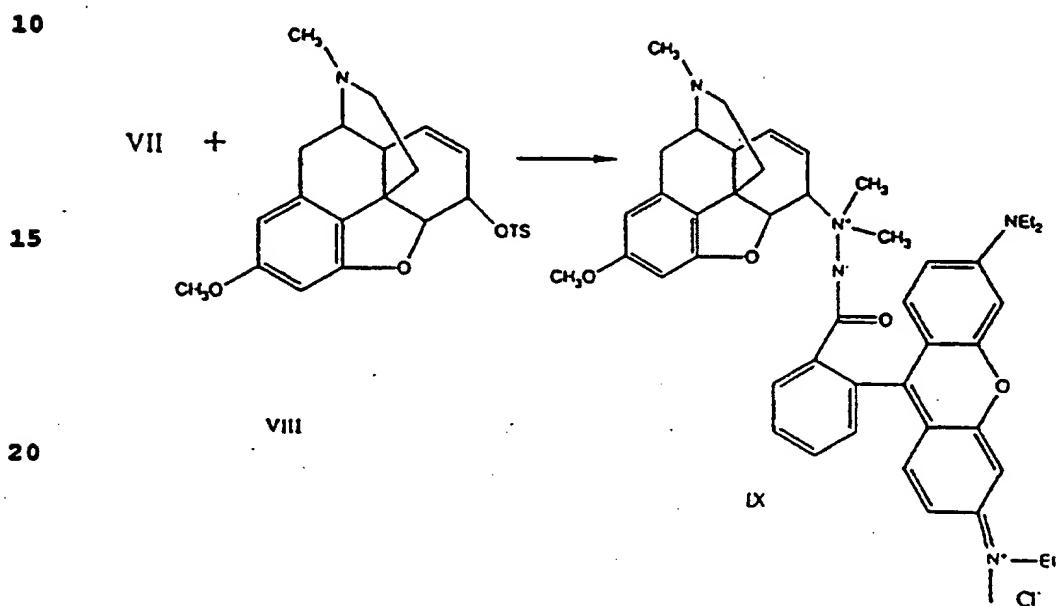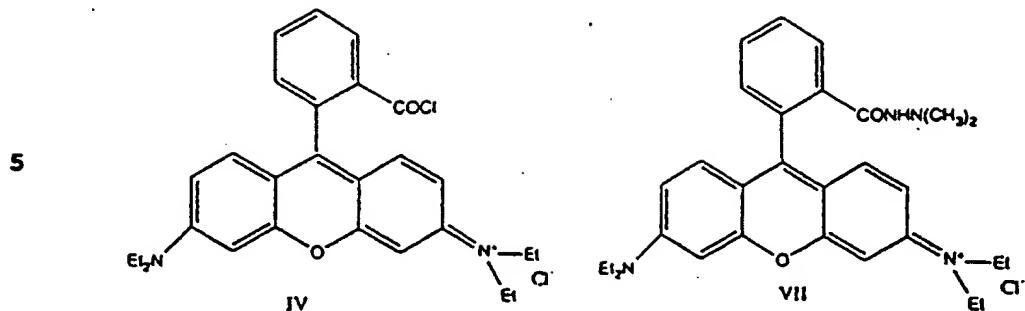
8.    EXAMPLE:  SYNTHESIS OF DISPERSE-BLUE-3-CONTAINING
      LIGAND MIMETIC USEFUL IN THE ISOLATION AND
      PURIFICATION OF CODEINE-BINDING PROTEINS

     To a solution of 0.285 g (0.001 mol) of norcodeine
(X) dissolved in 50 ml of a suitable solvent (such as
5   benzene) is added a solution of 0.139 g (0.001 mol) of
4,4'-dimethylvinylazlactone (XI) in 10 ml of the same
solvent and the resulting solution is heated to 70 °C and
held at this temperature for 10 hrs.  At the end of this
time the temperature is brought to 10 °C with cooling and
10  0.06 g (0.001 mol) of 1,1-dimethylhydrazine dissolved in
10 ml of the same solvent is added dropwise.  The
solution is then re-heated to 70 °C and held at this
temperature for 2 hours.  0.466 g (0.001 mol) of the
Disperse blue 3 tosylate (XII), prepared by the standard
15  tosylation techniques from a pure sample of the dye
(obtained from the commercial material by standard
normal-phase silica chromatography), is added and the
mixture is heated at 70 °C for 2 more hours.  The solvent
is then removed *in vacuo*, the residue is redissolved in
20  an appropriate alcohol solvent and titrated to pH 8
(measured with moist pH paper) with 10% (w/v) methanolic
KOH.  The precipitated salts are then removed by
filtration, and the filtrate is stripped *in vacuo* to give
conjugate (XIII), useful as a probe for the location and
25  isolation of receptor proteins that bind codeine and
similar molecules.



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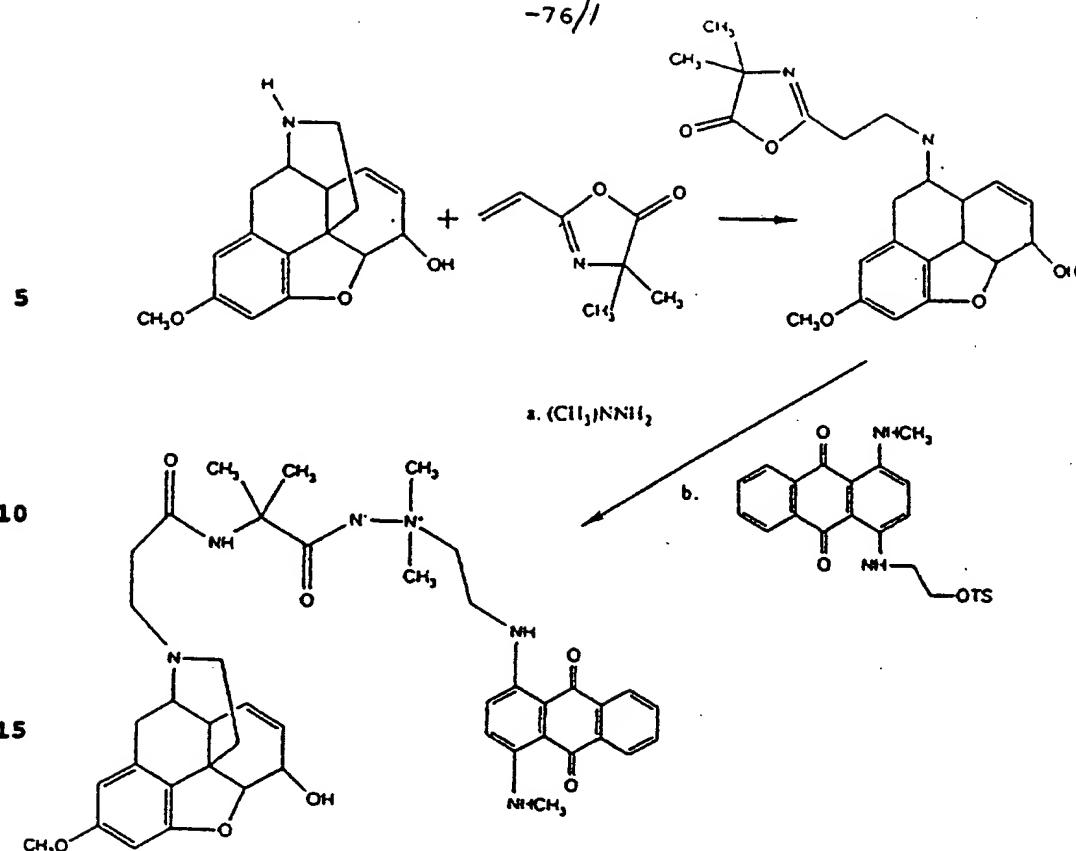




9.    EXAMPLE:   SYNTHESIS OF AN AMPHIPHILIC LIGAND
      MIMETIC FOR THE ISOLATION AND PURIFICATION OF
      CODEINE-BINDING PROTEINS

          29.95 g (0.1 mol) of octadecylisocyanate is
added slowly to 6.01 g (0.1 mol) of 1,1-dimethylhydrazine
in 100 ml of benzene.  The mixture is stirred for 18
hours at room temperature and 54.2 g (0.1 mol) of tosyl
codeine (VIII), prepared by the standard techniques, is
added portionwise over a 1/2-hour period.  The mixture is
heated to reflux and stirred at reflux for 2 hours.  At
the end of this time the solvent is removed in vacuo, the
residue is dissolved in an appropriate solvent (such as
ethanol), and the pH is titrated to 8 (measured with
moist pH paper) with 10% (w/v) methanolic KOH.  The
precipitated salts are then removed by filtration and the
solvent is removed in vacuo to give the crude conjugate
(XIV), useful for stabilizing and isolating receptor
proteins that bind to codeine and to similar molecules.

-77-



XIV
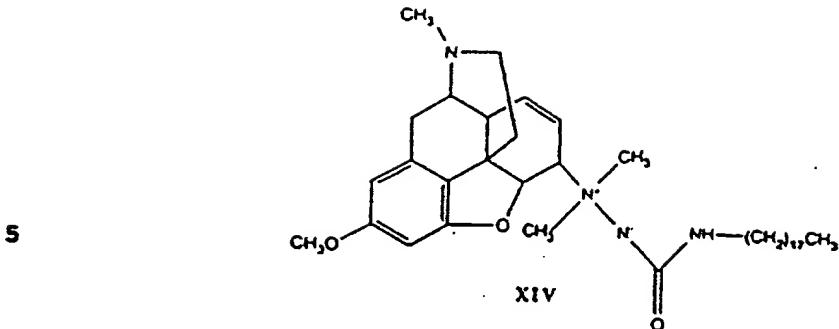
## 10.  EXAMPLE:  SYNTHESIS OF A MIMETIC OF THE PROTEIN-KINASE BINDING PEPTIDE

a.  The dodecamer peptide (BEAD)-Asp-His-Ile-Ala-Asn-Arg-Arg-Gly-Thr-Arg-Gly-Ser-NH$_2$ is obtained attached to the solid support as shown using standard FMOC peptide synthesis techniques, after deprotection of the terminal FMOC group.  This peptide is shaken with a solution of an equivalent molar amount of ClCH$_2$COCl in a suitable solvent at 50 °C for 6 hours.  The solvent is removed by decantation, leaving a terminal -NH-CO-CH$_2$Cl group attached to the peptide.

b.  A solution of equimolar amounts of 1,1-dimethylhydrazine and dicyclohexylcarbodiimide in a suitable solvent is treated with an equivalent molar amount of the heptamer peptide H$_2$N-Thr-Thr-Tyr-Ala-Asp-Phe-Ile-COOH, prepared and obtained in the free state using the standard FMOC solid phase peptide synthesis chemistry (e.g., using instruments and methods marketed by the Milligen Division of Millipore Corp.).  The mixture is stirred for 4 hours at room temperature.  The precipitated N,N'-dicyclohexyl urea is removed by centrifuging and decantation, and the solution is added to the functionalized beads prepared in a. above.  The mixture is then heated to 50 °C and shaken overnight at this temperature.  The mixture is cooled, the solvent removed by decantation, and the peptide released from the bead to yield the aminimide mimetic H$_2$N-Thr-Thr-Tyr-Ala-Asp-Phe-Ile-CO-N-N(CH$_3$)$_2$-CH$_2$-Ser-Gly-Arg-Thr-Gly-Arg-Asn-

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Ala-Ile-His-Asp-COOH. This mimetic has the aminimide in place of alanine in the naturally occurring protein-kinase binding peptide, UK (5-24), and is useful as a synthetic binding peptide with enhanced proteolytic stability.

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11.1.  **SYNTHESIS OF A MIMETIC OF A HUMAN ELASTASE INHIBITOR**

This example teaches the synthesis of a competitive inhibitor for human elastase based on the
10  structure of known N-trifluoroacetyl dipeptide analide inhibitors (see 162 J. Mol. Biol. 645 (1982) and references cited therein).

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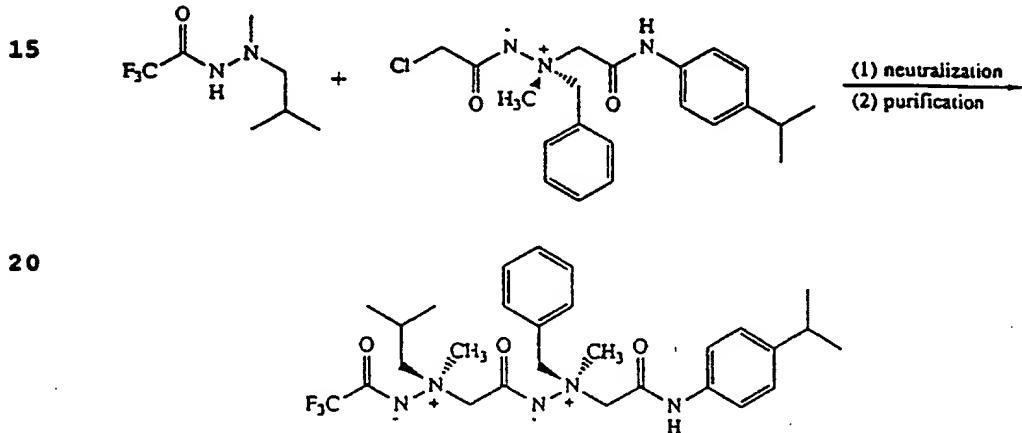
To 3.7 g (0.01 mol) of the aminimide N-(p-isopropylanalido)-methyl)-S-N-methyl-N-
30  benzylchloromethylacetamide in 50 ml ethanol was added 1.86 g (0.01 mol) of 1-methyl-1-isobutyl-2-N-trifluoroacetyl hydrazone (prepared from the reaction of trifluoroacetic anhydride with 1-methyl-1-isobutylhydrazine [from methylisobutylamine and
35  chloramine] using standard acylation methods) in 50 ml

ethanol.  The mixture was heated to reflux and stirred at reflux for 4 hours.  The mixture was then cooled to room temperature and titrated with 10% (w/v) KOH in methanol to the phenolphthalein endpoint.  The mixture was then filtered and the solvent removed *in vacuo* on a rotary

5  evaporator.  The residue was taken up in benzene and filtered.  Removal of the benzene on the rotary evaporator yielded 5.1 g (95%) of crude mixed diastereomeric aminimides.  The desired (S)-(S) isomer was obtained by normal-phase chromatographic purification

10  over silica.  This product is useful as a competitive inhibitor for human elastase, characterized by HPLC on Crownpack™ CR(+) chiral stationary phase (Daicell Chemical Industries Ltd.) using pH 2 aqueous mobile phase.  NMR (DMSO-$d_6$): Chemical shifts, peak integrations

15  and $D_2O$ exchange experiments diagnostic for structure.

11.2.        Synthesis of the Chiral
             Chloroaminimide Starting Material

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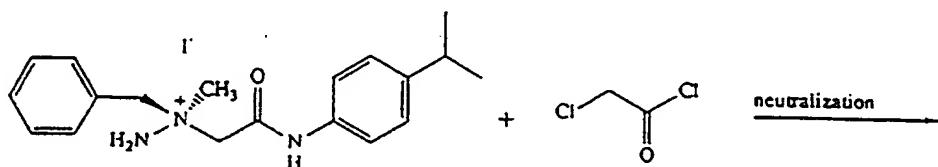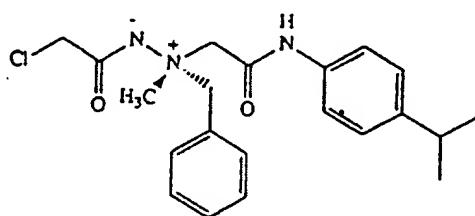
A mixture of 4.2 g (0.01 mol) of the
hydrazinium iodide enantiomer prepared as outlined below,
1.0 g (0.0106 mol) chloroacetic acid and 1.24 g (0.011
mol) chloracetyl chloride, contained in a micro reaction
flask equipped with a drying tube, was heated in an oil
bath at 105°C for 1 hour. The (homogeneous) reaction
mixture was then cooled to room temperature and extracted
with 4 x 20 ml of ethyl ether to remove chloracetyl
chloride and chloracetic acid, with vigorous stirring
each time. The residual semisolid was dissolved in the
minimum amount of methanol and titrated with 10% KOH in
methanol to the phenolphthalein end point. The
precipitated salts were filtered and the filtrate
evaporated to dryness on a rotary evaporator at 40°C.
The residue was taken up in benzene and filtered. The
solvent was removed on a rotary evaporator to yield 3.37
g (90%) of the (S)-aminimide enantiomer, characterized by
CDCL$_3$ NMR spectrum and D$_2$O exchange experiments and
directly used in the next step in the sequence (see
above).

11.3.     Synthesis of the Chiral
          Aminimide Starting Material



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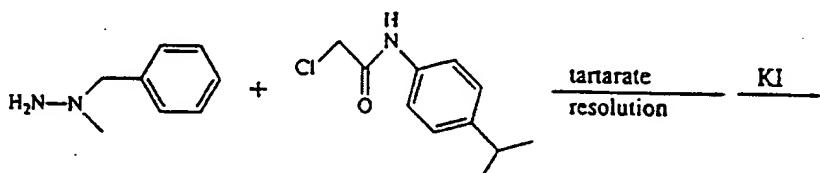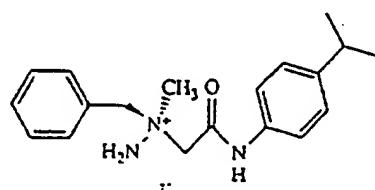
13.6 g (0.1 mol) of 1-methyl-1-benzyl-hydrazine (prepared from methyl benzyl amine and chloramine using standard methods [J. Chem. Ed. 485 (1959)]) in 125 ml of toluene was cooled to 5°C in an ice bath. To this was gradually added, with vigorous stirring over a one-hour period, a solution of 21.17 g (0.1 mol) of p-isopropylphenyl chloromethyl analide (prepared from chloracetyl chloride and p-isopropylphenyl amine) dissolved in 100 ml of toluene. Throughout the addition, the temperature was maintained at 5°C. The reaction mixture was then allowed to warm to room temperature, and was stirred overnight. The precipitated solid hydrazinium salt was filtered, washed with cold toluene and dried in a vacuum oven at 60°C/30" to yield 34.3 g (98%) of racemic product. This racemate was slurried at room temperature overnight in 100 ml ethanol and a slight molar excess of moist silver oxide was added and the mixture was stirred at room temperature overnight. The mixture was then filtered into an ethanolic solution containing an equivalent of D-tartaric acid in the minimum amount of solvent. The alcoholic filtrate was concentrated to approximately 20% of its volume and diethylether was added until turbidity was observed. The turbid solution was then cooled at 0°C overnight and the crystals were collected by filtration. The solid substance was purified by recrystallization from ethanol/ether to yield the desired pure diastereomeric salt, which was subsequently converted to the iodide form by precipitation from a water-ethanol solution of the tartrate (made alkaline by the addition of sodium carbonate) on treatment with an equivalent of solid potassium iodide, characterized by HPLC on Crownpack™ CR(+) chiral stationary phase (Daicell Chemical Industries Ltd.) using pH 2 aqueous mobile phase. NMR (DMSO-$d_6$): chemical shifts, peak integrations & $D_2O$ exchange experiments diagnostic for structure.

## 14. EXAMPLE: SYNTHESIS OF A PEPTIDE MIMETIC INHIBITING HUMAN ELASTASE
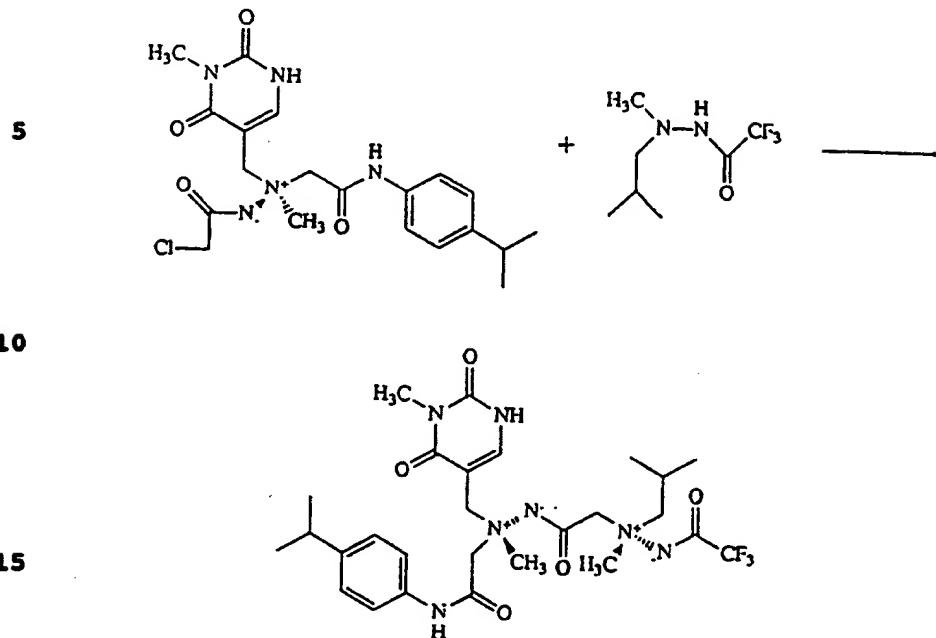


To 4.36 g (0.01 mol) of the chloromethylaminimide above in 50 ml ethanol was added a solution of 1.86 g (0.01 mol) of 1-methyl-1-isobutyl-2-N-trifluoroacetyl hydrazone (prepared from the reaction of trifluoroacetic anhydride with 1-methyl-1-isobutylhydrazine [from methyl isobutyl amine and chloramine] using standard acylation conditions) in 50 ml of ethanol. The mixture was heated to reflux, stirred at reflux for 4 hours, then cooled to room temperature and titrated with 10% (w/v) KOH in methanol to the phenolphthalein endpoint. The mixture was filtered and the solvent was removed *in vacuo* on a rotary evaporator. The residue was taken up in benzene and again filtered. Removal of the benzene on the rotary evaporator yielded 5.7 g (95%) of the mixed (R)-(S) and (S)-(S) aminimide diastereomers. The desired (S)-(S) isomer was obtained pure by normal-phase chromatographic purification over silica. This product is useful as a competitive inhibitor for human elastase, characterized

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by HPLC on Crownpack™ CR(+) chiral stationary phase
(Daicell Chemical Industries Ltd.) using pH 2 aqueous
mobile phase.  NMR (DMSO-$d_6$): chemical shifts, peak
integrations & $D_2O$ exchange experiments diagnostic for
structure.

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Synthesis of the Chiral Chloroaminimide

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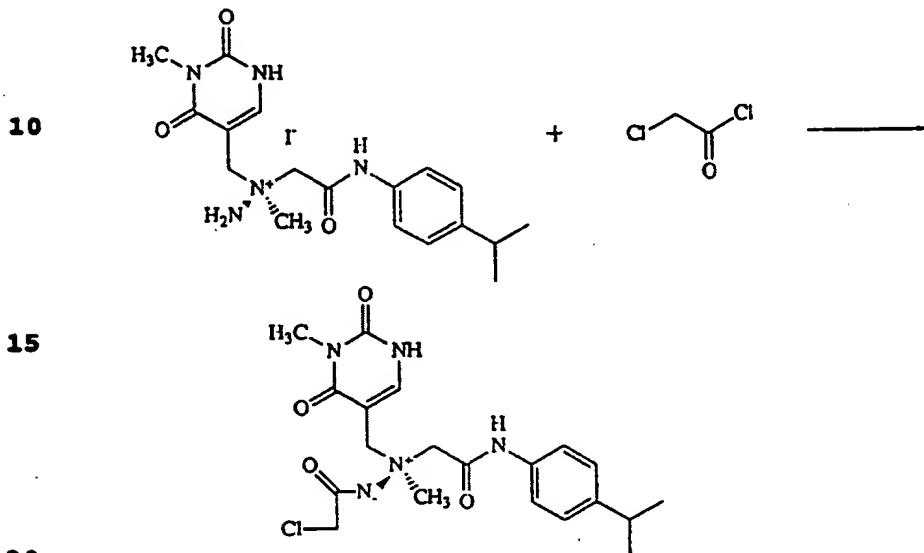
A mixture of 4.87 g (0.01 mol) of the
hydrazinium iodide enantiomer prepared as described in
5.2.3, 1.0 g (0.0106 mol) chloroacetic acid and 1.24 g
(0.011 mol) chloroacetyl chloride, contained in a micro
reaction flask equipped with a drying tube, was heated at
25   105°C for 1 hour with an oil bath.  The (homogeneous)
reaction mixture was then cooled to room temperature and
extracted with 4 x 20 ml of ethyl ether to remove
chloracetyl chloride and chloracetic acid.  The residual
semisolid mass was dissolved in the minimum amount of
30   methanol and titrated with 10% KOH in methanol to the
phenolphthalein end point.  The precipitated salts were
filtered and the filtrate was evaporated to dryness on a
rotary evaporator at 40°C.  The residue was then taken up
in benzene and filtered. The solvent was removed on a
35   rotary evaporator to give 3.88 g (89%) of th  (S)-

aminimide enantiomer, characterized by CDCL₃ NMR spectrum
and D₂O exchange experiments and used directly in the next
step in the synthesis (see above).

<u>Synthesis of the Chiral Aminimide</u>
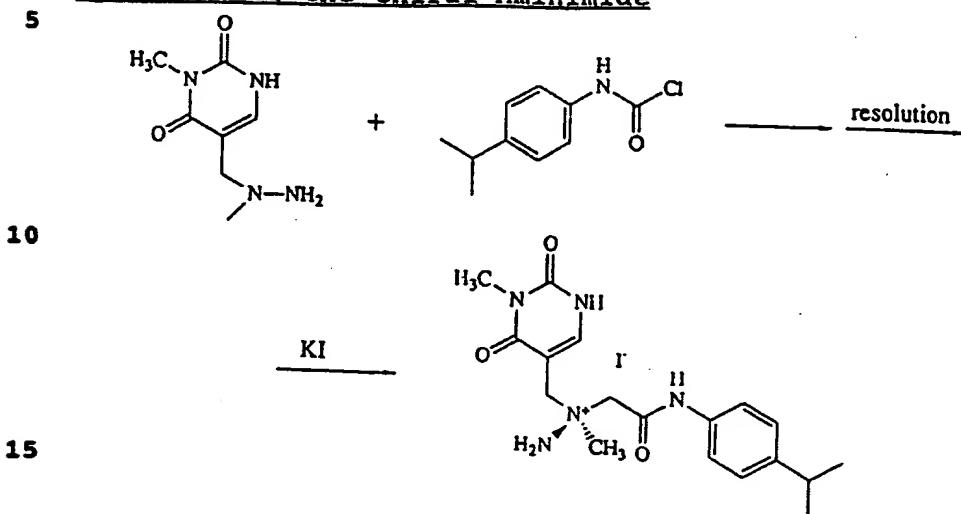


    18.4 g (0.1 mol) of 1-(5'[3'-methyl uracil]
methyl)-1-methylhydrazine (prepared by the alkylation of
2-methylphenylhydrazone with 5-chloromethyl-3-methyl
uracil in ethanol, as described in 24 <u>J. Org. Chem.</u> 660
(1959) and references cited therein, followed by removal
of the benzoyl group by acid hydrolysis) in 100 ml
toluene was cooled to 5°C in an ice bath and a solution
of 21.1 g (0.1 mol) p-isopropylphenyl-chloromethylanalide
(prepared from chloracetyl chloride and p-
isopropylanaline), in 100 ml of toluene, was added
thereto with vigorous stirring over a 1-hour period,
maintaining a temperature of 5°C. The reaction mixture
was then allowed to warm to room temperature and was
stirred overnight. The solution was cooled to 0°C and
the precipitated hydrazinium chloride salt was filtered,
washed with cold toluene and dried in a vacuum oven at
40°C/30" to yield 4.77 g (98%) of crude racemic product.
This racemate was slurried in 100 ml ethanol, a slight
molar excess of moist silver oxide was added, and the

mixture was stirred at room temperature vernight. This
racemate was resolved via its tartrate salts and isolated
as the iodide using the method of Singh, above,
characterized by HPLC on Crownpack™ CR(+) chiral
stationary phase (Daicell Chemical Industries Ltd.) using
5 pH 2 aqueous mobile phase. NMR (DMSO-$d_6$): chemical
shifts, peak integrations & $D_2O$ exchange experiments
diagnostic for structure.

Synthesis of 3-methyl-5-chloromethyluracil

10        A.    74.08 g (1 mol) of N-methyl urea and 216.2
g (1 mol) diethylethoxymethylenemalonate were heated
together at 122°C for 24 hours, followed by 170°C for 12
hours, to give the 3-methyluracil-5-carboxylic acid ethyl
ester in 35% yield (following recrystallization from
15 ethyl acetate).

          B.    30 g 3-methyluracil-5-carboxylic acid
ethyl ester was saponified with 10% NaOH to yield the
free acid in 92% yield (after standard work-up and
recrystallization from ethyl acetate).
20        C.    20 g of 3-methyluracil-5-carboxylic acid
was decarboxylated at 260°C to give a quantitative yield
of 3-methyluracil.

          D.    3-methyluracil-5-carboxylic acid was
treated with HCl and $CH_2$ using standard chloromethylation
25 conditions to give 3-methyl-5-chloromethyl uracil in 52%
yield (following standard work-up and recrystallization
from ethyl acetate).  NMR (DMSO-$d_6$): chemical
shifts, peak integrations & $D_2O$ exchange experiments
diagnostic for structure.
30

35

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13.   EXAMPLE:  SYNTHESIS OF A PEPTIDE
      MIMETIC INHIBITING THE HIV PROTEASE
          This example teaches the synthesis of a
competitive inhibitor for the HIV protease with enhanced
stability, based on the insertion of a chiral aminimide
5   residue into the scissile bond position of the substrate
Ac-L-Ser(Bzl)-L-Leu-L-Phe-L-Pro-L-Ile-L-Val-OMe (see,
e.g., 33 J. Med. Chem. 1285 (1990) and references cited
therein).

10
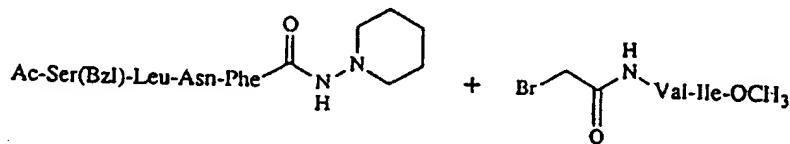


15
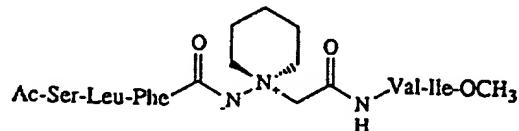
20        0.735 gms (1 mmol) of Ac-Ser(Bzl)-Leu-Asn-Phe-
CO-NH-NC$_5$H$_{10}$ is dissolved in the minimum amount of DMF, and
0.344 g of BrCH$_2$CONH-Val-Ile-OMe, prepared by treatment of
H$_2$N-Val-Ile-OMe with (BrCH$_2$CO)$_2$O according to the method of
Kent (256 Science 221 (1992), is added thereto.  The
25   mixture is heated to 60°C and stirred at this temperature
overnight.  At this point the DMF is removed under high
vacuum, and the desired (S) isomer is obtained from the
enantiomeric mixture after neutralization by standard
normal-phase silica chromatography to yield the protected
30   peptide.  The side chain blocking groups are subsequently
removed using standard peptide deprotection techniques to
yield the product Ac-Ser-Leu-Asn-Phe-CONN$^+$(C$_5$H$_{10}$)-CH$_2$-CO-
NH-Val-Ile-OMe, useful as a enhanced stability
competitive inhibitor for the HIV protease.
35

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Synthesis of the Tetrapeptide Hydrazone

Ac-Ser(Bzl)-Leu-Asn-Phe-OH  +  H₂N—N⟨piperidine⟩  ————→

**5**

Ac-Ser(Bzl)-Leu-Asn-Phe—N⟨piperidine⟩
                          H
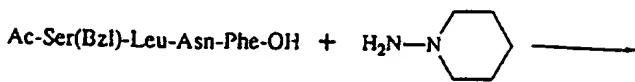
**10**        0.653 g (1 mmol) of AcSer(Bzl)-Leu-Asn-Phe-OH,
prepared via standard peptide synthesis techniques (see
33 J. Med. Chem. 1285 (1990) and references cited
therein), is coupled with 0.10 g (1 mmol) of 1-
aminopiperidine using standard peptide-coupling methods
**15** and chemistries (see 33 J. Org. Chem. 851 (1968)) to give
a 97% yield of the hydrazide, isolated by removal of the
reaction solvent *in vacuo*.

**20**   14.  EXAMPLE:  PREPARATION OF CHIRAL MONOMER
             USEFUL IN POLYMERIZATION YIELDING
             CROSSLINKED POLYMER-CHAINS

**25**   [chemical structures]

**30**   [chemical structure]
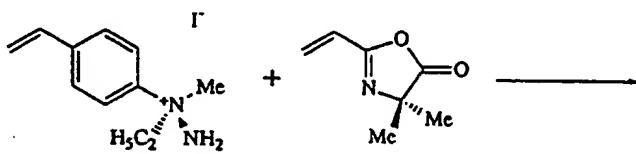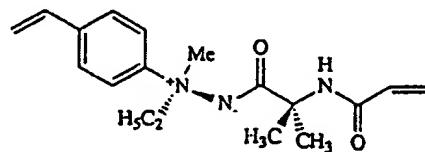
**35**        3.18 g (0.01 mol) of (S)-1-methyl-1-ethyl-1-p-
vinyl- benzylhydrazinium iodide, prepared from p-

vinylbenzyl chloride and 1-methyl-1-ethylhydrazine using
standard alkylation conditions, and isolated as the (S)-
enantiomer by the method of Singh (103 <u>J. Chem. Soc.</u> 604
(1913)), were added to 75 ml of anhydrous t-butanol. The
mixture was stirred under nitrogen and 1.12 g (0.01 mol)
5   of potassium t-butoxide was added. The mixture was
stirred for 24 hours at room temperature and the reaction
mixture was diluted with 75 ml of anhydrous THF, cooled
in an ice bath and 1.39 g (0.01 mol) of 2-vinyl-4,4-
dimethylazlactone in 50 ml of THF were then added over a
10  15-min. period. When addition was complete, the mixture
was allowed to warm to room temperature and stirred at
room temperature for 6 hours. The solvent was stripped
under aspirator vacuum on a rotary evaporator to yield
3.0 g (92%) of crude monomer. The product was
15  recrystallized from ethyl acetone at -30°C to yield pure
crystalline momomer, useful for fabricating crosslinked
chiral gels, beads, membranes and composites for chiral
separations, particularly for operation at high pH. NMR
(CDCl$_3$) chemical shifts, presence of vinyl groups in 6 ppm
20  region, vinyl splitting patterns, peak integrations and
D$_2$O experiments diagnostic for structure. FTIR absence of
azlactone CO band in 1820 cm$^{-1}$ region.


25




30




35

15. EXAMPLE: FUNCTIONALIZATION OF SILICA WITH AN OXAZOLONE FOLLOWED BY CONVERSION TO A CHIRAL AMINIMIDE USEFUL IN THE RESOLUTION OF RACEMIC CARBOXYLIC ACIDS

5



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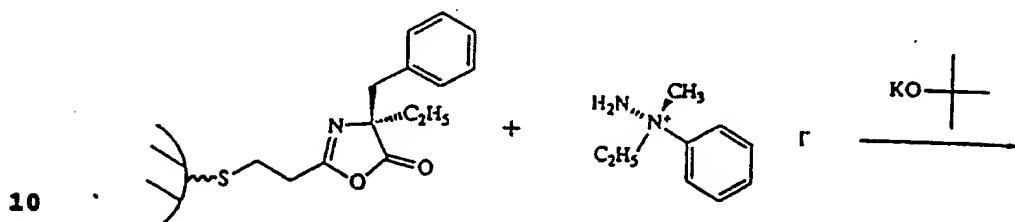
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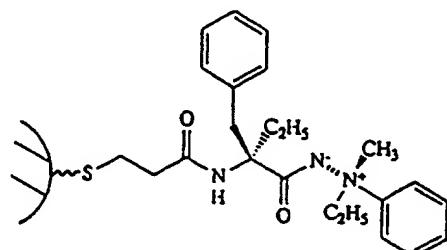
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    2.81 g (0.01 mol) of (S)-1-methyl-1-ethyl-1-
phenyl- hydrazinium iodide, prepared by the method of
Singh (103 J. Chem Soc. 604 (1913)), was added to 100 ml
anhydrous t-butanol. The mixture was stirred under
5   nitrogen and 1.12 g  (0.01 mol) potassium t-butoxide was
added. The mixture was stirred for 24 hours at room
temperature, after which the reaction mixture was diluted
with 100 ml anhydrous THF. To this mixture was added 5.0
g silica functionalized with the Michael-addition product
10  of (S)-4-ethyl-4-benzyl-2-vinyl- 5-oxazolone to
mercaptopropyl-functional silica. This mixture was
stirred at room temperature for 8 hours. The
functionalized silica was collected by filtration and
successively reslurried and refiltered using 100-ml
15  portions of toluene (twice), methanol (four times) and
water (twice). The resulting wet cake was dried in a
vacuum oven at 60°C under 30" vacuum to constant weight,
yielding 4.98 g of chiral-aminimide-functionalized
silica, useful for the separation of racemic mixtures of
20  carboxylic acids, such as ibuprofen, ketoprofen and the
like.


    16.  EXAMPLE:  FUNCTIONALIZATION OF SILICA WITH A CHIRAL
         AMINIMIDE FOR USE IN THE SEPARATION OF MANDELATES

25



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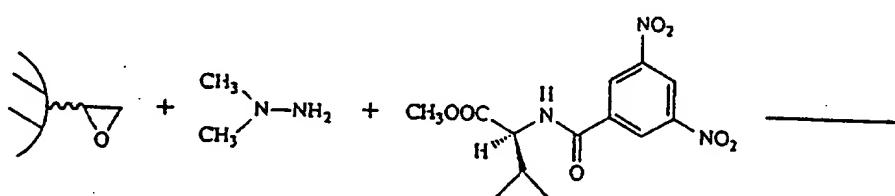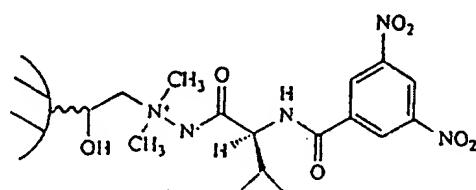
10.0 g epoxy silica (15μ Exsil C-200 silica) was slurried in 75 ml methanol and shaken to uniformly wet the surface. To this slurry was added 6.01 g (0.01 mol) 1,1-dimethylhydrazine, and the mixture was allowed

5   to stand at room temperature with periodic shaking for 45 min. 32.5 g (0.1 mol) of (S)-3,5-dinitrobenzoylvaline methyl ester was added and the mixture was allowed to stand at room temperature with periodic shaking for three days. The functionalized silica was then collected by

10  filtration, re-slurried in 100 ml methanol and re-filtered a total of five times, then dried in a vacuum oven at 60°C/30" overnight to give 9.68 g of the product. This functionalized silica was slurry packed from methanol into a 0.46 x 15 cm stainless steel column and

15  used to separate mixtures of mandelic acid derivatives under standard conditions.


Preparation of Epoxy Silica

        50 g of 5 μ C-200 Exsil silica (SA 250 $\mu^2$/g) was

20  added to 650 ml toluene in a two-liter three-necked round-bottomed flask equipped with a Teflon paddle stirrer, a thermometer and a vertical condenser set up with a Dean-Stark trap through a claisen adaptor. The slurry was stirred, heated to a bath temperature of 140°C

25  and the water was azeotropically removed by distillation and collection in the Dean-Stark trap. The loss in toluene volume was measured and compensated for by the addition of incremental dry toluene. 200 g of glycidoxypropyl trimethoxysilane was added carefully

30  through a funnel and the mixture was stirred and refluxed overnight with the bath temperature set at 140°C. The reaction mixture was then cooled to about 40°C. The resulting functionalized silica was collected on a Buechner filter, washed twice with 50 ml toluene, sucked

35  dry, reslurried in 500 ml toluene, refiltered, reslurried

in 500 ml methanol and refiltered a total of four times. The resulting methanol wet cake was dried overnight in a vacuum oven set for 30" at 60°C to yield 48.5 g of epoxy silica.

5  Synthesis of N-3,5-Dinitrobenzoyl-(S)-Valine Methyl Ester

10 
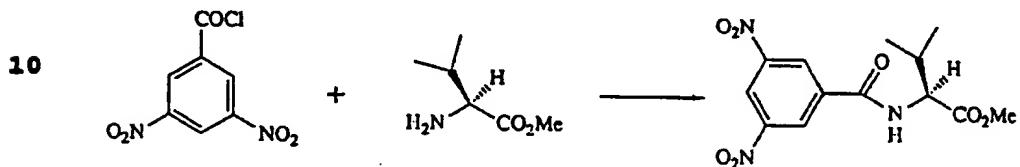
15      13.12 g (0.1 mol) of (S)-valine methyl ester was added with stirring to a solution of 8 g (0.2 mol) sodium hydroxide in 50 ml of water, cooled to about 10°C, and the mixture stirred at this temperature until complete solubilization was achieved.  23.1 g (0.1 mol)
20 of 3,5-dinitrobenzoylchloride was then added dropwise with stirring, keeping the temperature at 10-15°C with external cooling.  After the addition was complete, stirring was continued for 30 min.  To this solution was added over a 10-min. period 10.3 ml (1.25 mol) of
25 concentrated hydrochloric acid, again keeping the temperature at 15°C.  After this addition was complete, the reaction mixture was stirred for an additional 30 min. and cooled to 0°C.  The solid product was collected by filtration, washed well with ice water and pressed
30 firmly with a rubber dam.  The resulting wet cake was recrystallized from ethanol/water and dried in a vacuum oven under 30" vacuum at 60°C to yield 28.5 g (90%) of N-3,5-dinitrobenzoyl-(S)-valine methyl ester.  NMR (CDCl₃): chemical shifts, splitting patterns, integrations and D₂O
35 exchange experiments diagnostic for structure.

17.  EXAMPLE:  PREPARATION OF AMINIMIDE-CONTAINING
     ION-EXCHANGE SILICA MATRIX

          This example describes preparation of an
aminimide-functionalized ion-exchange silica matrix using
epoxy silica as the support to be modified.  The reaction
5  sequence is:


Epoxy Silica + $(CH_3)_2NNH_2$ + $Et_2NCH_2CH_2COOEt$ --->


$-\overset{|}{\underset{|}{Si}}-O-\overset{|}{\underset{|}{Si}}CH_2CH_2CH_2OCH_2CH(OH)CH_2N(CH_3)_2NCOCH_2CH_2NEt_2$
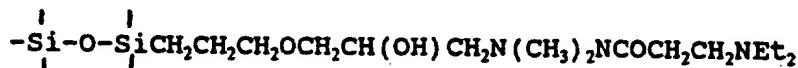
10

          25 g of epoxy silica (15$\mu$ Exsil AWP 300 silica,
with surface area of 100 $m^2/g$) was slurried in 100 ml
methanol until completely wetted by the solvent.  10.2 g
of 1,1-dimethylhydrazine were then added with swirling
15  and the mixture allowed to stand at room temperature for
3 hours.  24.7 g of $Et_2NCH_2CH_2COOEt$ were then added and the
mixture kept at room temperature with periodic shaking
for 2 days.
          The diethylaminoethyl (DEAE) functionalized
20  silica was collected by filtration, re-slurried in 100 ml
methanol and re-filtered a total of five times.  The
packing was dried in a vacuum oven at 60°C/30" overnight.
A 1.0 ml bed of this material was then packed in a 15 mM
NaAc buffer at pH 7.7.  The column was then equilibrated
25  with 15 mM NaAc buffer at pH 5.6, and a solution of 1
mg/ml ovalbumin in this buffer run through the bed at a
flow rate of 1.6 ml/min.  A total of 59.2 ml of protein
solution was run.
          The column was then washed with 41.7 ml of 15
30  mM NaAc buffer at pH 5.58 and at a flow rate of 3.9
ml/min.  The bound protein was eluted using 23.4 ml of
0.5M NaCl at a flow rate of 3.9 ml/min.  The eluent (15.2
ml) was then collected and the transmission of an aliquot
measured at 280 m$\mu$ with a spectrophotometer.  The
35  ovalbumin c ncentration was determined from a calibration

curve.  The total amount of ovalbumin collected was 63.7 mg.


18.   EXAMPLE:   PREPARATION OF AMINIMIDE-CONTAINING
      SIZE-EXCLUSION SILICA MATRIX

5           This example describes preparation of an
aminimide-functionalized size-exclusion silica matrix
using the epoxy silica support described above.

            10.0 g of epoxy silica (15μ Exsil C-200 silica,
with surface area of 250 $m^2$/g) was slurried in 75 ml of
10  methanol and shaken to uniformly wet the surface.  To
this slurry was added 10.2 g of 1,1-dimethylhydrazine.
The mixture was allowed to stand at room temperature with
periodic shaking for 45 min.

            15 g of ethyl acetate were then added and the
15  mixture allowed to stand at room temperature with
periodic shaking for 3 days.  The functionalized silica
was then collected by filtration, re-slurried in 100 ml
methanol, re-filtered a total of five times and dried in
a vacuum oven at 60°C/30" overnight.  The functionalized
20  silica was slurry packed from methanol into a 10mm
interior-diameter jacketed glass column with adjustable
pistons to provide an 8 cm-long packed bed.  This packing
was used to separate mixtures of polyethylene glycol
polymers of varying molecular weight with good resolution
25  using a mobile phase.

            In a second experiment, the bulk packing was
found to selectively adsorb polyethylene-glycol
functionalized hemoglobin from serum samples taken from
test animals that had been treated with this derivative
30  as a blood substitute.  Filtration of the serum, after
treatment with the bulk packing, gave a serum free from
the functionalized hemoglobin, thus allowing blood
screening or testing by means of standard methods.


35  19.   EXAMPLE:   COATING OF A SILICA MATRIX WITH
           HYDROXYPROPYL CELLULOSE FUNCTIONALIZED WITH

## AN AMINIMIDE

Hydroxypropylcellulose is mono-functionalized by reaction, under strong alkaline conditions (preferably provided by a strong base, such as potassium t-butoxide) with $ClCH_2CONN^+(CH_3)_3$. The result is replacement of
5  approximately one hydroxyl group in each saccharide unit with the aminimide as follows:

$$OCH_2CO\overset{-}{N}\overset{+}{N}(CH_3)_3$$
$$|$$
10               $[SACCHARIDE\ UNIT]_n$
$$|\qquad\qquad|$$
$$OH\qquad\quad OH$$
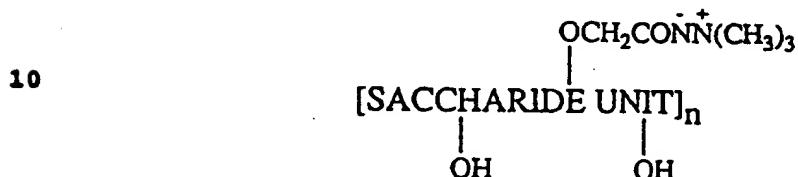
The resulting aminimide derivative is coated
15  onto a surface (e.g., silica). Upon heating to 140 °C, the $N(CH_3)_3$ group leaves, resulting in formation of an isocyanate moiety:

$$OCH_2NCO$$
$$|$$
20              $[SACCHARIDE\ UNIT]_n$
$$|\qquad\qquad|$$
$$OH\qquad\quad OH$$
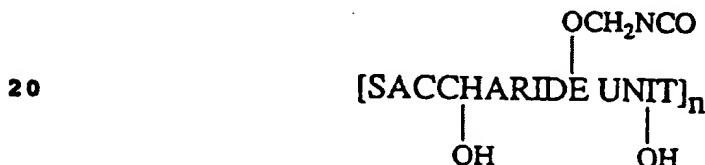
The isocyanate groups then react with unreacted
25  hydroxyl groups on the saccharide units to produce a cross-linked coating.

Alternatively, the cellulose can be coated onto the surface and immobilized using standard techniques (e.g., reaction with bisoxiranes), and then mono, di- or
30  tri-substituted with desired aminimide derivatives as described above.

The foregoing reaction sequence can also be employed with polymers or oligomers bearing NH or SH groups instead of hydroxyl groups and can also be
35  utilized to fabricate structures such as crosslinked cellulose membranes.

20.  EXAMPLE:  COATING OF A SILICA MATRIX VIA
POLYMERIZATION OF AN AMINIMIDE ON THE MATRIX

This example illustrates an alternative
immobilization technique, namely, polymerizing aminimide
precursors containing vinyl groups and which have been
5 coated onto a surface.  The chemistry resembles the
approach described above, except polymerization forms a
sturdy shell around an existing support rather than
creating a solid block of material.

This sequence makes use of the reaction
10 described above.  An epoxide,

$$(CH_3)_3-N^+-CH_2-CH-CH_2 \quad \overset{CH_3 \quad Cl^-}{\underset{CH_3}{}} \quad \overset{O}{\diagdown}$$

15
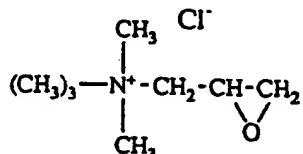
is combined with methyl methacrylate and
dimethylhydrazine as set forth in 2.a above to form
$CH=C(CH_3)-CO-NN(CH_3)_2-CH_2-CH(OH)-CH_2-N^+(CH_3)_3Cl^-$.  3.11 g of
20 this material and 0.598 g n-methylol acrylamide were
dissolved in 75 ml of methanol, and 3.54 ml of water were
then added.  To this solution were added 15 g of epoxy
silica (15$\mu$ Exsil AWP 300 silica, with surface area of
100 m$^2$/g).

25       The mixture was stirred in a rotary at room
temperature for 15 min and then stripped using a bath
temperature of 44 °C to a volatiles content of 15% as
measured by weight loss (from 25-200 °C with a sun gun).
The coated silica was slurried in 100 ml of isooctane
30 containing 86 mg of VAZO-64 dissolved in 1.5 ml toluene
which had been de-aerated with nitrogen.  The slurry was
thoroughly de-aerated wtih nitrogen and then stirred at
70 °C for two hours.

The c ated silica was collected by filtration
35 and washed three times in 100 ml methanol and air dried.

The silica was heated at 120 °C for 2 hours to cure the coating. 13.1 g of coated silica were obtained. A 1 ml bed of this material was packed in an adjustable glass column and successfully used to separate BSA from lactoglobulin.

## 21. EXAMPLE: PREPARATION OF SILICA SUPPORT <u>CONTAINING</u> <u>CROSSLINKED AMINIMIDE POLYMER CHAINS</u>

In this example, an epoxy-functionalized surface is reacted with disubstituted hydrazine, a bisepoxide and a triester to form a crosslinked network of aminimide chains attached covalently to the surface as follows:
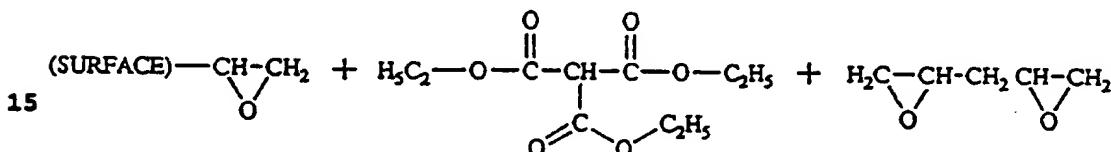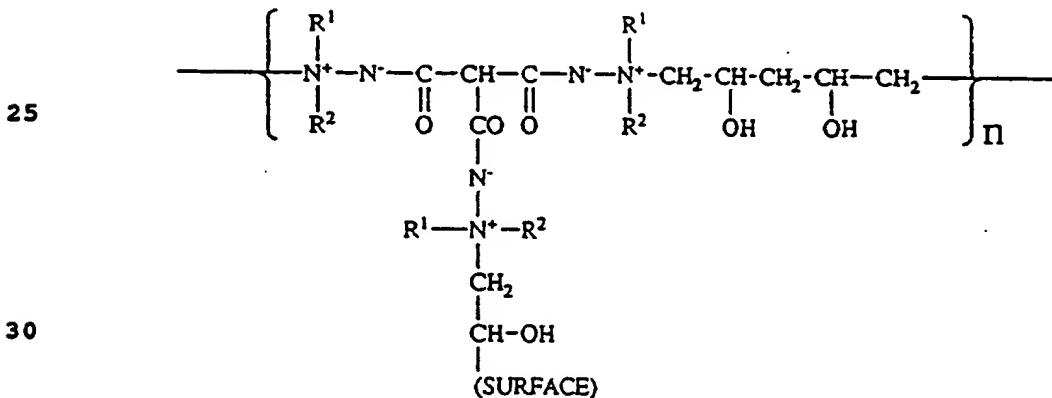
The reaction can be carried out in water at room temperature without special conditions.


22.  EXAMPLE:  PREPARATION OF CROSS-LINKED
5      POROUS AMINIMIDE ION-EXCHANGE BEADS

This example describes preparation of three-dimensional cross-linked porous copolymeric aminimide ion-exchange beads.  It involves reaction of three monomers:

10  Monomer A:      $CH_2=CH-CON^-N^+(CH_3)_3$

Monomer B:      $CH_2=C(CH_3)-CON^-N^+(CH_3)_2-CH_2-CH(OH)-CH_2-$
                $N^+(CH_3)_3Cl^-$

Crosslinker:    $CH_2=CH-CO-NH-C(CH_3)_2-CON^-N^+(CH_3)_2-CH_2-Ph-$
                $CH=CH_2$

15  where Ph is phenyl.

Preparation of Monomer A:  This monomer was prepared according to the method described in 21 J. Polymer Sci., Polymer Chem. Ed. 1159 (1983).

Preparation of Monomer B:  30.3 g (0.2 mol) of
20  glycidyl-trimethylammonium chloride was dissolved in 100 ml of methanol and filtered free of insolubles.  22 g (0.22 mol) of methyl methacrylate was added thereto, followed by 12 g (0.2 mol) of 1,1-dimethylhydrazine.  The solution grew warm and turned slightly pink.  It was
25  allowed to stand for 6 days at room temperature, and was then treated with charcoal, filtered, and concentrated on a rotary evaporator at 55 °C and 10mm to produce a thick lavendar-colored, viscous material.  This material was triturated with diethylether and hot benzene and
30  dissolved in the minimum amount of methanol.  The mixture was then treated with charcoal, filtered, heated to boiling and brought to the cloud point with ethyl acetate.  The resulting solution was allowed to stand at 0 °C for a week.  The white crystals that f rmed were
35  coll cted by filtration, washed with cold ethyl acetate

and dried in a vacuum oven at room temperature to yield
7.3 g of monomer B.

Preparation of Monomer C:   18 g (0.3 mol) of 1,1-
dimethylhydrazine was dissolved in 50 ml $CH_2Cl_2$ and cooled
in an ice bath with stirring.  41.7 g (0.3 mol) of
$^5$ vinylazlactone in 50 ml $CH_2Cl_2$ were added slowly to keep
the temperature below 5 °C.  The clear solution was
stirred and allowed to come to room temperature over 1
hour (resulting in formation of a white solid) and was
stirred at room temperature for an additional 1.5 hours.
$^{10}$ The white solid was collected by filtration, re-slurried
in 100 ml $CH_2Cl_2$ and re-filtered.  It was then dried in a
vacuum oven at room temperature overnight to yield a
total of 26.81 g of the intermediate
$CH_2=CH-CO-NH-C(CH_3)_2-CO-NH-N-(CH_3)_2$.  10.0 g (0.05 mol) of
$^{15}$ this intermediate and 7.66 g (0.05 mol) of vinyl benzyl
chloride were dissolved in a mixture of 50 ml ethanol and
50 ml $CH_3CN$.  The solution was refluxed for 4 hours under
a nitrogen stream.  It was then cooled to room
temperature and concentrated on a rotary evaporator at 55
$^{20}$ °C to produce a thick yellow oil.  The oil was triturated
three times with diethylether to yield 17.08 g of an off-
white solid.  This solid was dissolved in 100 ml of hot
methanol and filtered through a celite pad to remove a
small amount of gelatinous material, and the clear
$^{25}$ filtrate was stripped to yield 10.0 g of Monomer C as a
white solid.

Polymerization:   1 ml of the emulsifier Span 80 and
175 ml of mineral oil were introduced into a 500 ml
round-bottomed flask equipped with stirrer and a heating
$^{30}$ bath.  The mixture was mechanically stirred at 70 RPM and
brought to a temperature of 55 °C.  40.5 g of monomer A,
7.2 g of monomer B and 5.7 g of the cross-linker were
dissolved in 100 ml of demineralized water and heated to
55 °C.  To this solution were added 150 mg of ammonium
$^{35}$ persulfate, and the mixtur  was then poured into the

stirred mineral oil.  The agitation was adjusted to
produce a stable emulsion with an average droplet
diameter of approximately 75 μ (as determined with an
optical microscope).

5      After 15 min, 0.15 ml of TMED was added and
stirring was continued for an additional 45 min.  The
reaction mixture was cooled and allowed to stand
overnight.  The supernatant mineral oil phase was removed
by aspiration and the beads were collected by
decantation.  The beads were washed three times with a
10     0.05% solution of Triton X-100 in demineralized water to
remove any remaining mineral oil and then washed with
water and allowed to settle.  The water was removed by
decantation.

       This procedure was repeated a total of five
15     times.  The beads obtained at the conclusion of the
foregoing steps had a mean diameter of approximately 75 μ
and an ion-exchange capacity of 175 μeq/ml.


       23.    EXAMPLE:  PREPARATION OF AN AMINIMIDE-BASED
20            ELECTROPHORETIC GEL
              This example describes preparation of an
aminimide electrophoresis gel.  As a control, the
standard Sigma protein  electrophoresis mix (available
from Sigma Chemical Co., St. Louis, MO) was run on an
25     acrylamide/methylene bisacrylamide linear gradient gel
prepared using a gradient maker with 5% and 12.5% monomer
solutions, as shown below.  The gel was overlayed with
isobutanol and allowed to polymerize overnight.


| 30 Monomer | 5% Monomer | 12.5% |
|---|---|---|
| Lower Tris | 5.0 ml | 5.0 ml |
| H₂O | 11.7 ml | 4.7 ml |
| 35  30% Acrylamide | 3.3 ml | 8.3 ml |

| Glycerol | --- | 2.0 ml |
|---|---|---|
| Ammonium Persulfate | 30 μl | 30 μl |
| TMED | 15 μl | 15 μl |

Lower Tris 1.5M:  6.06 g Tris base, 8 ml 10% SDS, volume adjusted to 90 ml with double-distilled water. The pH was adjusted to 6.0 with concentrated HCl, and the final volume adjusted to 100 ml with DD water.

Acrylamide 30% w/v:  29.2 g acrylamide, 0.8 g of methylene bisacrylamide and 100 ml DD water.

SDS 10% w/v:  10 g of SDS dissolved in DD water and adjusted to a volume of 100 ml.

Ammonium persulfate 10%:  0.1 g ammonium persulfate was dissolved in 0.9 ml DD water.  The solution was used within 4 hours of preparation.

TMED:  used directly as obtained from Sigma Chemical Co., St. Louis, MO, under the tradename TMEDA.

A second gel was prepared by replacing the acrylamide with an equal weight of the aminimide monomer $CH_2=CH-CO-N-N(CH_3)_3$, and the protein standard was run in the same way as the first.

Separation of proteins with the aminimide gel were equivalent to the acrylamide gel, but the aminimide gel produced Rf (i.e., the ratio of distance traversed by a particular protein to the distance traversed by the solvent front) levels approximately 20% higher than those of the acrylamide gel.

24.   EXAMPLE:  PREPARATION OF AMINIMIDE-BASED
        LATEX PARTICLES

This example describes preparation of latex particles containing an aminimide comonomer.

591.1 ml of distilled water was charged to a three-necked round-bottomed flask.  A nitrogen dip tube was placed below the liquid level and the nitrogen flow rate set to 2 cm³/min.  The solution was mechanically

agitated with a Teflon paddle at 250 RPM and heated to 80
°C over a half-hour period. In a separate flask were
dissolved 121.6 g of butyl acrylate, 54.6 g of ethyl
acrylate, 13.0 g of acrylic acid, 9.97 g of methyl
methacrylate, 59.7 g of the aminimide monomer $CH_2=CH-CO-N-$

5   $N(CH_3)_2-CH_2-CH_2-OH$ and 0.92 g of Aerosol TR-70 so as to
obtain solution without exceeding a temperature of 25 °C.
When completely dissolved, 1.53 g of additional TR-70
were added and the mixture was then stirred until
solution was achieved.

10       20.7 ml of distilled water was purged with
nitrogen for 10 min and 1.59 g of $K_2S_2O_8$ is dissolved in
it. This persulfate solution was added to the heated
water in the reaction flask after it stabilized at 80 °C.
The nitrogen dip tube was raised and a nitrogen blanket

15  was maintained. The monomer mix was pumped in at a
steady, calibrated rate such that the constant addition
took exactly 4 hours. When addition was complete, the
latex was post-heated at 80 °C for 1 hour, cooled to 25
°C and titrated to pH 5.0 by dropwise addition of

20  triethylamine (approximately 20 cm³) over 20 min with
agitation. The latex was then filtered through cheese
cloth and stored. Average particle size was measured at
about 0.14μ.

       It should be apparent to those skilled in the

25  art that other compositions and processes for preparing
the compositions not specifically disclosed in the
instant specification are, nevertheless, contemplated
thereby. Such other compositions and processes are
considered to be within the scope and spirit of the

30  present invention. Hence, the invention should not be
limited by the description of the specific embodiments
dislosed herein but only by the following claims.


35

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## THE CLAIMS

What is claimed is:

1.    A composition having the structure

$$A-X-\left[CO-N^{-}-\overset{\overset{\displaystyle R^{1..n}}{|}}{N^{+}}-G^{1..n}\right]_{n}-Y-B$$

wherein

a.    A and B are the same or different, and each is a chemical bond; hydrogen; an electrophilic group; a nucleophilic group; R; an amino acid derivative; a nucleotide derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component, wherein A and B are optionally connected to each other or to other structures and R is as defined below;

b.    X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c.    R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d.    G is a chemical bond or a connecting group that includes a terminal carbon atom for attachment to the quaternary nitrogen and G may be different in adjacent n units; and

e.    $n \geq 1$;

provided that, (1) if G is a chemical bond, Y includes a
terminal carbon atom for attachment to the quaternary
nitrogen; and (2) if n is 1, X and Y are chemical bonds
and R and R' are the same, A and B are different and one
is other than H or R.

2.    The composition of claim 1 wherein n > 2.

3.    The composition of claim 1 wherein at least one
of R and R' includes a hydroxyl containing substituent.

4.    The composition of claim 1 wherein G includes
at least one of an aromatic ring, a heterocyclic ring, a
carbocyclic moiety, an alkyl group or a substituted
derivative thereof.

5.    The composition of claim 1 wherein A and B are
the same.

6.    The composition of claim 1 where R and R' are
different so that the composition is chiral.

7.    The composition of claim 1 wherein at least one
of A and B is a terminal-structural moiety of formula T-
U, wherein:

        a.    U is selected from the group
        consisting of aliphatic chains having from    2
        to 6 carbon atoms, substituted or unsubstituted
        aryl, substituted or unsubstituted cycloalkyl,
        and substituted or unsubstituted heterocyclic
        rings; and

        b.    T is selected from the group
        consisting of $-OH$, $-NH_2$, $-SH$, $(CH_3)_3N^+-$,        $-$
        $SO_3-$, $-COO^-$, $CH_3$, H and phenyl.

8.    The composition of claim 1 wherein at least one
of A and B is $HO-CH_2-(CHOH)_n-$.

9.    The composition of claim 1 wherein A and B are
part of the same cyclic moeity.

10.   A peptide mimetic having the structure

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$$A—X \left\{ CO—N^-—N^+ \genfrac{}{}{0pt}{}{R^{1...n}}{|}{\underset{|}{\overset{|}{R'^{1...n}}}} G^{1...n} \right\}_n Y—B$$

wherein:

a.   A and B are the same or different, and at least one is an amino acid derivative of the form $(AA)_m$, wherein AA is a natural or synthetic amino acid residue and m is an integer, and A and B are optionally connected to each other or to other structures;

b.   X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c.   R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d.   G is a chemical bond or a connecting group that includes a terminal carbon atom for attachment to the quaternary nitrogen and G may be different in adjacent n units; and

e.   $n \geq 1$;

provided that, (1) if G is a chemical bond, Y includes a terminal carbon atom for attachment to the quaternary nitrogen; and (2) if n is 1, X and Y are chemical bonds and R and R' are the same, A and B are different and one is other than H or R.

11.   A nucleotide mimetic having the structure:

**SUBSTITUTE SHEET**

$$A—X—\left[\left(CO—N^{-}—\overset{\overset{\displaystyle R^{1...n}}{|}}{\underset{\underset{\displaystyle R'^{1...n}}{|}}{N^{+}}}—G^{1...n}\right)\right]_{n}—Y—B$$
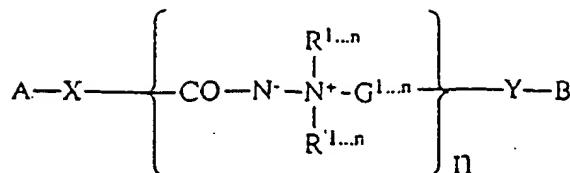
wherein:

5          a.    A and B are the same or different,
        and at least one is a nucleotide derivative,
        wherein A and B are optionally connected to
        each other or to other structures;

           b.    X and Y are the same or different and
10       each represents a chemical bond or one or more
        atoms of carbon, nitrogen, sulfur, oxygen or
        combinations thereof;

           c.    R and R' are the same or different
        and each is an alkyl, cycloalkyl, aryl, aralkyl
15       or alkaryl group or a substituted or
        heterocyclic derivative thereof, wherein R and
        R' may be different in adjacent n units and
        have a selected stereochemical arrangement
        about the carbon atom to which they are
20       attached;

           d.    G is a chemical bond or a connecting
        group that includes a terminal carbon atom for
        attachment to the quaternary nitrogen and G may
        be different in adjacent n units; and

25          e.    $n \geq 1$;
        provided that, (1) if G is a chemical bond, Y includes a
        terminal carbon atom for attachment to the quaternary
        nitrogen; and (2) if n is 1, X and Y are chemical bonds
        and R and R' are the same, A and B are different and one
30       is other than H or R.

           12.    The nucleotide mimetic of claim 11 wherein A is
        a nucleotide derivate of the form $(NUCL)_l$, wherein l is an
        integer, such that $(NUCL)_l$ is a natural or synthetic
        nucleotides when l=1, a nucleotide probes when l=2-25 and
35       an oligonucleotides when l>25 including both deoxyribose
        (DNA) and ribose (RNA) variants.

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13.   A carbohydrate mimetic having the structure:

$$
A\text{—}X\underbrace{\left[\; \text{CO—N}^{\cdot}\text{—}\underset{\underset{R'^{1\ldots n}}{|}}{\overset{\overset{R^{1\ldots n}}{|}}{N^{+}}}\text{-}G^{1\ldots n}\;\right]_{n}}\text{—}Y\text{—}B
$$
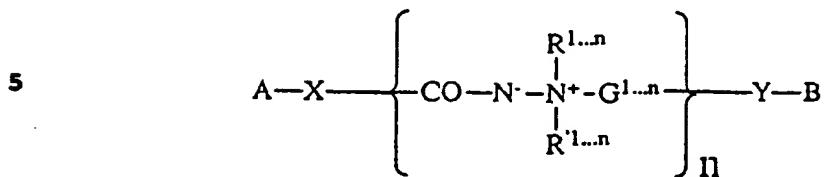
wherein:

a.   A and B are the same or different,
and at least one is a carbohydrate derivative;
wherein A and B are optionally connected to
each other or to other structures;

b.   X and Y are the same or different and
each represents a chemical bond or one or more
atoms of carbon, nitrogen, sulfur, oxygen or
combinations thereof;

c.   R and R' are the same or different
and each is an alkyl, cycloalkyl, aryl, aralkyl
or alkaryl group or a substituted or
heterocyclic derivative thereof, wherein R and
R' may be different in adjacent n units and
have a selected stereochemical arrangement
about the carbon atom to which they are
attached;

d.   G is a chemical bond or a connecting
group that includes a terminal carbon atom for
attachment to the quaternary nitrogen and G may
be different in adjacent n units; and

e.   $n \geq 1$;

provided that, (1) if G is a chemical bond, Y includes a
terminal carbon atom for attachment to the quaternary
nitrogen; and (2) if n is 1, X and Y are chemical bonds
and R and R' are the same, A and B are different and one
is other than H or R.

14. The carbohydrate mimetic of claim 13 wherein A and B each is a natural carbohydrate, a synthetic carbohydrate residue or derivative thereof or a related organic acid thereof.

15. A pharmaceutical compound having the structure:

$$A—X—\left[—CO—N^--\overset{\overset{\textstyle R^{1..n}}{|}}{N^+}-G^{1..n}—\right]_n—Y—B$$
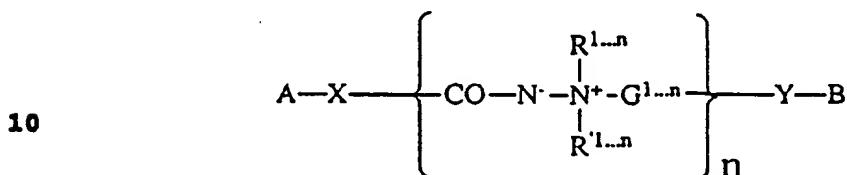
wherein:

    a.    A and B are the same or different, and at least one is an organic structural motif; wherein A and B are optionally connected to each other or to other structures;

    b.    X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

    c.    R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

    d.    G is a chemical bond or a connecting group that includes a terminal carbon atom for attachment to the quaternary nitrogen and G may be different in adjacent n units; and

    e.    $n \geq 1$;

provided that, (1) if G is a chemical bond, Y includes a terminal carbon atom for attachment to the quaternary

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nitrogen; and (2) if n is 1, X and Y are chemical bonds and R and R' are the same, A and B are different and one is other than H or R.

    16.   The pharmaceutical compound of claim 15 wherein the structural motif of the organic compound is obtained from a pharmaceutical compound or a pharmacophore or metabolite thereof and has specific binding properties to ligands.

    17.   A reporter compound having the structure:

$$A-X-\left[-CO-N^{-}-\overset{\overset{\displaystyle R^{1...n}}{|}}{N^{+}}-G^{1...n}-\right]_{n}-Y-B$$
$$\underset{R'^{1...n}}{|}$$
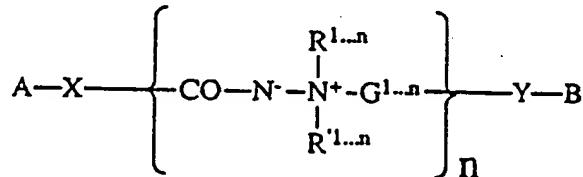
wherein:

    a.   A and B are the same or different, and at least one is a reporter element; wherein A and B are optionally connected to each other or to other structures;

    b.   X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

    c.   R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

    d.   G is a chemical bond or a connecting group that includes a terminal carbon atom for

attachment to the quaternary nitrogen and G may
be different in adjacent n units; and

     e.   $n \geq 1$;

provided that, (1) if G is a chemical bond, Y includes a
terminal carbon atom for attachment to the quaternary
nitrogen; and (2) if n is 1, X and Y are chemical bonds
and R and R' are the same, A and B are different and one
is other than H or R.

     18.   The reporter compound of claim 17 wherein the
reporter element is a natural or synthetic dye or a
photographically active residues which possesses reactive
groups which may be synthetically incorporated into the
oxazolone structure or reaction scheme and may be
attached through the groups without adversely interfering
with the reporting functionality of the group.

     19.   The reporter compound of claim 17 wherein the
reactive group is amino, thio, hydroxy, carboxylic acid,
acid chloride, isocyanate alkyl halide, aryl halide or an
oxirane group.

     20.   A polymer having the structure:

$$A-X-\left[CO-N^{-}-\overset{\overset{\displaystyle R^{1...n}}{|}}{\underset{\underset{\displaystyle R'^{1...n}}{|}}{N^{+}}}-G^{1...n}\right]_{n}-Y-B$$
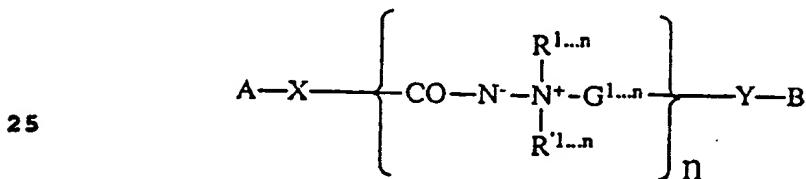
wherein:

     a.   A and B are the same or different,
and at least one is an organic moiety
containing a polymerizable group; wherein A and
B are optionally connected to each other or to
other structures;

     b.   X and Y are the same or different and
each repr sents a chemical bond or one or more

at ms of carbon, nitrogen, sulfur, oxygen  r
combinations thereof;

    c.    R and R' are the same or different
and each is an alkyl, cycloalkyl, aryl, aralkyl
or alkaryl group or a substituted or
heterocyclic derivative thereof, wherein R and
R' may be different in adjacent n units and
have a selected stereochemical arrangement
about the carbon atom to which they are
attached;

    d.    G is a chemical bond or a connecting
group that includes a terminal carbon atom for
attachment to the quaternary nitrogen and G may
be different in adjacent n units; and

    e.    $n \geq 1$;
provided that, (1) if G is a chemical bond, Y includes a
terminal carbon atom for attachment to the quaternary
nitrogen; and (2) if n is 1, X and Y are chemical bonds
and R and R' are the same, A and B are different and one
is other than H or R.

    21.    The polymer of claim 20 wherein the
polymerizable group of the organic moiety is a vinyl
group, oxirane group, carboxylic acid, acid chloride,
ester, amide, lactone or lactam.

    22.    A substrate having the structure:

$$A - X - \left[ CO - N^- - \overset{\displaystyle R^{1...n}}{\underset{\displaystyle R'^{1...n}}{\overset{|}{\underset{|}{N^+}}}} - G^{1...n} \right]_n - Y - B$$
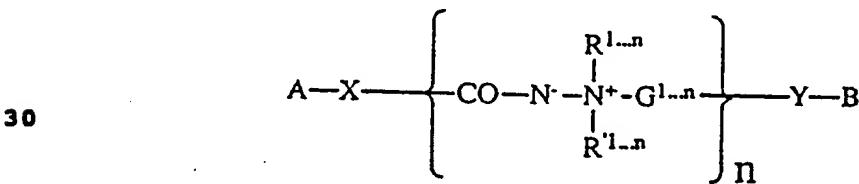
wherein:

    a.    A and B are the same or different,
and at least one is a macromolecular c mponent,

wherein A and B are optionally connected to
each other or to other structures;

   b.    X and Y are the same or different and
each represents a chemical bond or one or more
atoms of carbon, nitrogen, sulfur, oxygen or
combinations thereof;

   c.    R and R' are the same or different
and each is an alkyl, cycloalkyl, aryl, aralkyl
or alkaryl group or a substituted or
heterocyclic derivative thereof, wherein R and
R' may be different in adjacent n units and
have a selected stereochemical arrangement
about the carbon atom to which they are
attached;

   d.    G is a chemical bond or a connecting
group that includes a terminal carbon atom for
attachment to the quaternary nitrogen and G may
be different in adjacent n units; and

   e.    $n \geq 1$;
provided that, (1) if G is a chemical bond, Y includes a
terminal carbon atom for attachment to the quaternary
nitrogen; and (2) if n is 1, X and Y are chemical bonds
and R and R' are the same, A and B are different and one
is other than H or R.

   23.   The substrate of claim 21 wherein the
macromolecular component is a surface or structures which
is attached to the oxazolone module via a reactive group
in a manner where the binding of the attached species to
a ligand-receptor molecule is not adversely affected and
the interactive activity of the attached functionality is
determined or limited by the macromolecule.

   24.   The substrate of claim 23 wherein the
macromolecule component has a molecular weight of at
least about 1000 Daltons.

   25.   The substrate of claim 24 wherein the molecular
component is in the form of an ceramic particle, a

nanoparticle, a latex particle, a porous or non-por us
beads, a membrane, a gel, a macroscopic surface or a
functionalized or coated version or composite thereof.

    26.  A chiral composition of matter having the
structure

$$A—Y—G—\overset{\displaystyle R}{\underset{\displaystyle R'}{|\quad|}}N^+-N—W$$
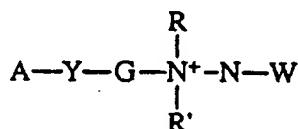
wherein

    a.   A is a chemical bond; hydrogen; an
electrophilic group; a nucleophilic group; R;
an amino acid derivative; a nucleotide
derivative; a carbohydrate derivative; an
organic structural motif; a reporter element;
an organic moiety containing a polymerizable
group; or a macromolecular component, wherein R
is as defined below;

    b.   Y represents a chemical bond or one
or more atoms of carbon, nitrogen, sulfur,
oxygen or combinations thereof;

    c.   W is -H⁻ or -H₂ X⁻ where X⁻ is an anion;

    d.  R and R' are the same or different
and each is an alkyl, cycloalkyl, aryl, aralkyl
or alkaryl group or a substituted or
heterocyclic derivative thereof, wherein R and
R' may be different in adjacent n units and
have a selected stereochemical arrangement
about the carbon atom to which they are
attached; and

    e.   G is a chemical bond or a connecting
group that includes a terminal carbon atom for
attachment to the quaternary nitrogen; provided
that if G is a chemical bond, Y includes a

terminal carbon atom for attachment to the
quaternary nitrogen.

27.  The composition of claim 26 wherein X is a
halogen or tosyl anion.

28.  The composition of claim 26 wherein A is a
terminal-structural moiety of formula T-U, wherein:

        a.  U is selected from the group
consisting of aliphatic chains having from 2 to
6 carbon atoms, substituted or unsubstituted
aryl, substituted or unsubstituted cycloalkyl,
and substituted or unsubstituted heterocyclic
rings; and

        b.  T is selected from the group
consisting of -OH, -NH$_2$, -SH, (CH$_3$)$_3$N$^+$-,
-SO$_3$-, -COO$^-$, CH$_3$, H and phenyl.

29.  The composition of claim 27 wherein A is
HO-CH$_2$-(CHOH)$_n$-.

30.  The composition of claim 26 where R and R' are
different so that the composition is chiral.

31.  The composition of claim 26 wherein Y is a
chemical bond, G is

$$\begin{array}{c} R \\ | \\ -C- \\ | \\ R' \end{array}$$

and A is -COO$^-$ or -COOR and W is -H$^-$, where R and R' differ
from each other and are as described above.

32.  The composition of claim 26 wherein Y is a
chemical bond, G is

$$\begin{array}{c} R \\ | \\ -C- \\ | \\ R' \end{array}$$
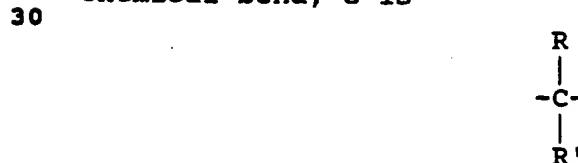
and A is -COO$^-$ or -COOR and W is -H$_2$ X$^-$, where R and R'
differ from each other and are as described above.

33.   A process of synthesizing a chiral c mposition
having the structure

$$A-X-\left\{ CO-N^{\cdot}-N^{+}-G^{1...n}\begin{array}{c}R^{1...n}\\|\\|\\R'^{1...n}\end{array}\right\}_n -Y-B$$

wherein

a.    A and B are the same or different,
and each is a chemical bond; hydrogen; an
electrophilic group; a nucleophilic group; R;
an amino acid derivative; a nucleotide
derivative; a carbohydrate derivative; an
organic structural motif; a reporter element;
an organic moiety containing a polymerizable
group; or a macromolecular component, wherein A
and B are optionally connected to each other or
to other structures and R is as defined below;

b.    X and Y are the same or different and
each represents a chemical bond or one or more
atoms of carbon, nitrogen, sulfur, oxygen or
combinations thereof;

c.    R and R' are the same or different
and each is an alkyl, cycloalkyl, aryl, aralkyl
or alkaryl group or a substituted or
heterocyclic derivative thereof, wherein R and
R' may be different in adjacent n units and
have a selected stereochemical arrangement
about the carbon atom to which they are
attached;

d.    G is a chemical bond or a connecting
group that includes a terminal carbon atom for
attachment to the quaternary nitrogen and G may
be different in adjacent n units; and

e.    n $\geq$ 1;

provided that, (1) if G is a chemical bond, Y includes a
terminal carbon atom for attachment to the quaternary

nitrogen; and (2) if n is 1, X and Y are chemical bonds
and R and R' are the same, A and B are different and one
is other than H or R;
wherein the process comprises the steps of:

5      acylating an asymmetric hydrazinium salt with a
molecule capable of functioning both as an acylating and
as an alkylating agent to form an aminimide;

reacting the aminimide with an asymmetrically
disubstituted hydrazine to form a diastereomeric mixture
10  of aminimide-hydrazinium salts.

34.   The process of claim 33 which further
comprises:

resolving the diastereomeric mixture and isolating a
selected diastereomer;

15      acylating the diastereomer with a second molecule
capable of functioning both as an acylating and as an
alkylating agent to form an aminimide;

capping the resulting aminimide; and

repeating the preceding steps at least once, if
20  necessary, to form the desired structure.

35.   The process of claim 33 wherein the asymmetric
hydrazinium salt is bound to a support surface.

36.   A process of synthesizing a chiral composition
having the structure

25
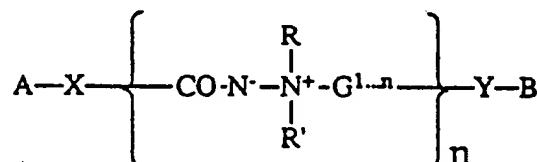
$$A-X\left[-CO\text{-}N^{-}\text{-}N^{+}\text{-}G^{1-n}-\right]_n Y-B$$
$$\overset{R}{\underset{R'}{|}}$$

30
wherein

a.   A and B are the same or different,
and each is a chemical bond; hydrogen; an
electrophilic group; a nucleophilic group; R;
35      an amino acid derivative; a nucleotide
derivative; a carbohydrate derivative; an

organic structural motif; a reporter element;
an organic moiety containing a polymerizable
group; or a macromolecular component, wherein A
and B are optionally connected to each other or
5      to other structures and R is as defined below;
       b.   X and Y are the same or different and
each represents a chemical bond or one or more
atoms of carbon, nitrogen, sulfur, oxygen or
combinations thereof;
10       c.   R and R' are the same or different
and each is an alkyl, cycloalkyl, aryl, aralkyl
or alkaryl group or a substituted or
heterocyclic derivative thereof, wherein R and
R' may be different in adjacent n units and
15      have a selected stereochemical arrangement
about the carbon atom to which they are
attached;
       d.   G is a chemical bond or a connecting
group that includes a terminal carbon atom for
20      attachment to the quaternary nitrogen and G may
be different in adjacent n units; and
       e.   n ≥ 1;
provided that, (1) if G is a chemical bond, Y includes a
terminal carbon atom for attachment to the quaternary
25   nitrogen; and (2) if n is 1, X and Y are chemical bonds
and R and R' are the same, A and B are different and one
is other than H or R;
wherein the process comprises the steps of:
       alkylating an asymmetrically disubstituted acyl
30   hydrazide with a molecule capable of functioning both as
an acylating and as an alkylating agent to form a racemic
mixture of aminimide isomers; and
       reacting the racemic mixture with an asymmetrically
disubstituted hydrazin  to form a racemic mixture of
35   aminimide-acyl hydrazide isomers.

37.  The process of claim 36 which further
comprises:
    resolving the mixture of aminimide-acyl hydrazide
isomers to isolate a desired isomer;
5    reacting the isolated isomer with a monofunctional
alkylating agent to produce an aminimide;  and
    capping the aminimide.
38.  The process of claim 36 which further
comprises:
10    reacting the mixture of aminimide-acyl hydrazide
isomers with a second molecule capable of functioning
both as an acylating and as an alkylating agent to form a
racemic mixture of aminimide isomers;
    repeating the preceding steps at least once, if
15 necessary, to form the desired structure.
39.  The process of claim 36 wherein the
asymmetrically disubstituted acyl hydrazide is bound to a
support surface.
40.  A composition prepared according to the process
20 of any one of claims 33 to 39.
41.  A lipid mimetic composition having the
structure:
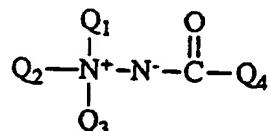
$$Q_2-\overset{\overset{\displaystyle Q_1}{|}}{\underset{\underset{\displaystyle Q_3}{|}}{N^+}}-N^--\overset{\overset{\displaystyle O}{\|}}{C}-Q_4$$

25

wherein Q is a chemical bond; an electrophilic
group; a nucleophilic group; R; an amino acid derivative;
a nucleotide derivative; a carbohydrate derivative; an
30 organic structural motif; a reporter element; an organic
moiety containing a polymerizable group; a macromolecular
component; or the substituent X(T) or X(T)$_2$; wherein R is
an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a
substituted or heterocyclic derivative thereof, and T is
35 a linear or branched hydrocarbon having between 12 and 20
carbon atoms some of which are optionally substituted

- 118 -

with oxygen, nitrogen  r sulfur atoms or by an aromatic
ring; and provided that at least two T substituents are
present in the structure of the composition.

42.  The composition of claim 41 wherein at least
one Q is attached to the α-carbon of a naturally
occurring amino acid, or at least one Q is a
carbohydrate.

43.  A functionalized polymer having the structure:

$$\text{(SURFACE)-CH-CH}_2 \left[ \begin{matrix} R^1 \\ | \\ N^+\text{-}N^-\text{-}C\text{-}X\text{-}C\text{-}N^+\text{-}NCH_2CH\text{-}Y\text{-}CH\text{-}CH \\ | \quad \| \quad \| \quad | \quad | \quad | \\ R^1 \quad O \quad O \quad R^2 \quad OH \quad OH \end{matrix} \right]_n \begin{matrix} R^{2n+1} \\ | \\ \text{-}N^+\text{-}N^-\text{-}C\text{-}X\text{-}C\text{-}OR \\ | \quad \| \quad \| \\ R^{2n+1} \quad O \quad O \end{matrix}$$
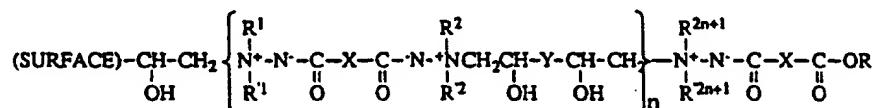
wherein

a.   X and Y are connecting groups;

b.   $R^a$ or $R'^a$ (where n = an integer) each represent
     hydrogen, alkyl, cycloalkyl, aryl, aralkyl and
     alkaryl;

c.   (Surface) is a macromolecular component; and

d.   n ≥ 1.

44.  A functionalized polymer having the formula:

$$\text{(SURFACE)}\text{-}\underset{\underset{O}{\|}}{C}\text{---} \left[ \begin{matrix} R^1 \\ | \\ \text{-}N\text{-}N^+\text{-}CH_2\,CH\text{-}Y\text{-}CH\,CH_2\text{-}^+N\text{-}\text{-}N\text{-}C\text{-}X\text{-}C \\ | \quad\quad | \quad | \quad | \quad \| \quad \| \\ R^1 \quad OH \quad OH \quad R^2 \quad O \quad O \end{matrix} \right]_n \text{---OR}$$
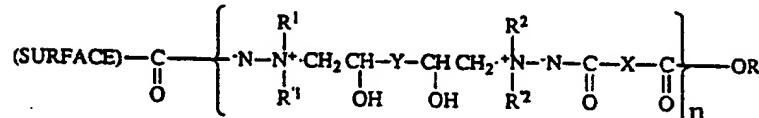
wherein

a.   X and Y are connecting groups;

b.   $R^a$ or $R'^a$ (where n = an integer) each represent
     alkyl, cycloalkyl, aryl, aralkyl and alkaryl;

c.   (Surface) is a macromolecular component; and

d.   n ≥ 1.

45.  A method of producing an aminimide-functional
support comprising the st ps of:

reacting a polymer or oligomer containing pendant
moieties of OH, NH or SH with a compound of the formula:

**5**

$$Cl\overset{O}{\underset{}{\diagdown}}N\overset{N}{\underset{R^3}{\diagdown}}\overset{R^1}{\underset{R^2}{\diagup}}$$
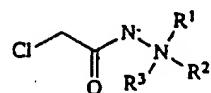
**10**                wherein $R^1$ and $R^2$ each represent alkyl,
cycloalkyl, aryl, aralkyl or alkaryl, and $R^3$ is
an amino acid derivative; a nucleotide
derivative; a carbohydrate derivative; an
organic structural motif; a reporter element;
**15**                an organic moiety containing a polymerizable
group; or a macromolecular component;
      b.     coating the reacted polymer or oligomer onto a
support to form a film thereon; and
      c.     heating the coated support to crosslink the
**20**              film.

      46.    A method of producing an aminimide-functional
support comprising the steps of:

coating a mixture of multifunctional esters and
multifunctional epoxides onto a support to form a film
**25** thereon; and

reacting the coated support with 1,1'-
dialkylhydrazine to crosslink the film.

      47.    A method of producing an aminimide-functional
support comprising the steps of:

**30**            coating a mixture of an aminimide-functional vinyl
monomer, a difunctional vinyl monomer and a vinyl
polymerization initiator onto a support to form a film
thereon; and

heating the coating support to form a crosslinked
**35** film.

48. An aminimide-functionalized support prepared according to the method of one of claims 45, 46 or 47.

49. A three-dimensional crosslinked random copolymer containing, in copolymerized form:

about 1 to 99 parts of a free-radically polymerizable monomer containing an aminimide group;

up to 98 parts of a free-radically addition-polymerizable comonomer; and

about 1 to 50 parts of at least one crosslinking monomer.

50. The copolymer of claim 49 wherein the comonomer is water-soluble.

51. The copolymer of claim 50 wherein the comonomer is water-insoluble.

52. The copolymer of claim 50 wherein the copolymer is fashioned into a water-insoluble bead, a water-insoluble membrane or a latex particle.

53. The copolymer of claim 50 wherein the copolymer is a swollen aqueous gel suitable for use as an electrophoresis gel.

54. A three-dimensional crosslinked random copolymer that is the reaction product of:

about 1 to 99 parts of a condensation-polymerizable monomer containing a moiety cluster selected from the group consisting of (1) at least three epoxy groups, (2) at least three ester groups, (3) at least one epoxy and at least two ester groups and (4) at least one ester and at least two epoxy groups;

about 1 to 99 parts of a second condensation-polymerizable monomer containing a moiety cluster selected from the group consisting of (1) at least two ester groups, (2) at least two epoxy groups and (3) at least one ester and one epoxy group; and

an amount of 1,1-dialkylhydrazine equivalent, on a molar basis, substantially equal to the total molar cont nt of epoxy groups.

55.  The copolymer of claim 54 wherein the copolymer
is fashioned into a water-insoluble bead, a water-
insoluble membrane or a latex particle.

56.  The copolymer of claim 55 wherein the the
5  copolymer is a swollen aqueous gel suitable for use as an
electrophoresis gel.


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# INTERNATIONAL SEARCH REPORT

| A. CLASSIFICATION OF SUBJECT MATTER |
|---|
| IPC(5) :A61K 31/205 |
| US CL :514/1 |
| According to International Patent Classification (IPC) or to both national classification and IPC |

| B. FIELDS SEARCHED |
|---|
| Minimum documentation searched (classification system followed by classification symbols) |
| U.S. : Please See Extra Sheet. |

| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched |
|---|
| |

| Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) |
|---|
| Please See Extra Sheet. |

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US, A, 4,260,705 (TSIBRIS) 07 APRIL 1981 Column 4, lines 1-40; Column 7; and Example 1, columns 8-9. | 1, 3, 4, 7, 20-22 |
| X | US, A, 3,671, 473 (SEDOR) 20 JUNE 1972 Column 4, line 65ff:equation(1) and column 5-6, (equation 2). | 1-4, 6, 7, 20-22 |
| X | US, A, 3,898,087 (BRUTCHEN) 05 AUGUST 1975 Column 3, lines 5-28. | 1, 3, 4, 7, 8, 20-22 |
| X | THE CHEMICAL SOCIETY OF JAPAN, "Synthesis, Surface-active Properties and Phase Transfer Catalytic Action of Crown Ethers Bearing Aminimide Group" (INOKUMA) Page 1495, schemes 1 and 2, 28 April 1983. | 1, 3, 4, 6-8 |

| [X] Further documents are listed in the continuation of Box C. | [ ] See patent family annex. |
|---|---|

| * Special categories of cited documents: | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
|---|---|
| "A" document defining the general state of the art which is not considered to be part of particular relevance | |
| "E" earlier document published on or after the international filing date | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "O" document referring to an oral disclosure, use, exhibition or other means | |
| "P" document published prior to the international filing date but later than the priority date claimed | "&" document member of the same patent family |

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 12 OCTOBER 1993 | 15 DEC 1993 |

| Name and mailing address of the ISA/US | Authorized officer |
|---|---|
| Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 | MARK NAGUMO |
| Facsimile No. NOT APPLICABLE | Telephone No. (703) 308-2351 |
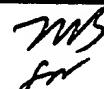
Form PCT/ISA/210 (second sheet)(July 1992)*

| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|---|---|---|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | JOURNAL OF THE CHEMICAL SOCIETY, PERKINS TRANSACTIONS II, 1983, "On the Nature of Nitrogen-Nitrogen Bonding in Cyclic Aminimides" (TSUCHIYA) pages 887-890. See Table 1, Compounds 2, 4-8. | 1, 9 |
| X | US, A, 3,985,807 (GRIMM) 12 OCTOBER 1976, Column 1, 2:structures 1-3, columns 5-6:Table 3, column 6: reaction formula, Columns 9-10: Table 5. | 1, 3, 4, 6, 7, 20-22 |
| X | US, A, 4,016,340 (KOLESINSKI) 05 APRIL 1977 Columns 2, 3, and 5-6. | 1, 4, 6, 7, 13, 20-22 |
| X | US, A, 4,330,443 (RANKIN) 18 MAY 1982, Column 2, line 50 to column 4, line 25. | 1, 4, 6, 7, 13, 20-22 |
| X | US, A, 3,949, 140 (BIEFELD) 06 APRIL 1976, Columns 3-5. | 1-7, 9, 20-22 |
| X | US, A, 3,946,131 (BIEFELD) 23 MARCH 1976, Column 3, line 55 to column 9, line 47 | 1, 3, 4, 7, 22 |

**Box I    Observations where certain claims were f und unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II    Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

  Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
   1-14, 20-25, 41

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark   n Protest**    ☐ .  The additional search fees were accompanied by the applicant's protest.

☒  No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)*

## B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

428/378, 411.1, 454; 514/44; 523/213; 526/292.95, 293, 304, 305, 307, 310, 312; 527/200, 312; 528/44, 45, 113, 332; 530/323, 345; 536/23.1, 53; 564/147

## B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CA
Terms-?aminimide?
amino acid, ?peptide?, oligopeptide?, polypeptide?
nucleotide?, ?nucleotide, oligonucleotide?, polynucleotide?
carbohydrate?, ?saccharide?, oligosaccharide?, polysaccharide?
starch, dextran
polymer?,
substrate
cycl?

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I*, claims 1-9, drawn to a composition, classified in Class 514, subclass 1.
Group II, Claim 10, drawn to a peptide mimetic, classified in Class 530, subclass 300.
Group III, Claims 11-12, drawn to nucleotide mimetics, classified in Class 536, subclass 22.
Group IV, Claims 13-14, drawn to carbohydrate mimetics, classified in Class 527, subclass 200.
Group V, Claims 15-16, drawn to pharmaceutical compounds, classified in Class 514, subclass 1.
Group VI, Claims 17-19, drawn to reporter compounds, classified in Class 549, subclass 200.
Group VII, Claims 20-21, drawn to "polymers", classified in Class 526, subclass 264.
Group VIII, Claims 22-25, drawn to substrates, classified in Class 428, subclass 454.
Group IX*, Claims 26-32, drawn to a chiral composition, classified in Class 514, subclass 2.
Group X*, Claims 33-35, drawn to processes of synthesizing a chiral composition, classified in Class 514, subclass 2.
Group XI*, Claims 36-40, drawn to processes of synthesizing a chiral composition, classified in Class 514, subclass 2.
Group XII*, Claims 41-42, drawn to lipid mimetic compositions, classified in Class 536, subclass 53.
Group XIII, Claim 43, drawn to a functionalized polymer (from ester functional group), classified in Class 525, subclass 326.1.Group XIV, Claim 44, drawn to a functionalized polymer (from epoxide functional group), classified in Class 525, subclass 326.1.
Group XV*, Claim 45 and 48/45, drawn to processes of making aminimide functionalized supports (bearing OH, SH, or NH reactive functions) and product thereof, classified in Class 427, subclass 372.2.
Group XVI, Claims 46 and 48/46, drawn to processes of making aminimide functionalized supports (bearing esters or epoxides), classified in Class 427, subclass 340.
Group XVII, Claim 47 and 48/47, drawn to processes of making aminimide functionalized supports from ethylenically unsaturated monomers, classified in Class 427, subclass 340.
Group XVIII, Claims 49-53, drawn to random free-radical addition copolymer, classified in Class 524, subclass 555.
Group XIX, Claims 55-56, drawn to a crosslinked random condensation polymer and aqueous gel thereof, classified in Class 524, subclass 598.
Numerous species of the invention exist, as set forth below:

The Groups marked "*", (Groups I, IX, X, XI, XV) recite the list, for (a) A and B, "an amino acid derivative; a nucleotide derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component," which correspond to Groups II, III, IV, V, VII, VIII, respectively: these are considered to be independent inventions for the reasonsgiven below. These claims further recite, as identities of A and B, the list " a chemical bond; hydrogen; an electrophilic group; a nucleophilic group; R," each term of which, to the extent that it is distinct from the others, defines a family of five patentably distinct inventions (four extra). In part (b), independently of the identities of A and B, the linking groups X and Y (" a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen, or combinations thereof") establish three further patentably distinct inventions, C, N, (O,S) (two extra). Part (c), to the extent that R and R' are substituted by 'non-functional' groups, defines only one inventive concept (zero extra). In part (d), G=chemical bond is clearly a distinct inventive concept from a physical connecting group (one extra). In part (e), the monoaminimides (n=1) are further distinct from poly-aminimides (n>1), as indicated by the limitations on X, Y, R, R', A and B (one extra).

Groups II-VIII recite the limitations (b)-(e), and so are drawn to the same number of inventions established by these limitations as in Group I.

The total number of extra inventions, not counting combinations, is considered to be $18+8=26$.

The first recited invention, to which Applicant is entitled to a search, is Group I, wherein A and B are HO-CH2-(CHOH)n-(limitations of claims 5, (7) and (8), and where $n>2$ (claim 2), and where R and R' are different, at least one of which contains -OH (claims 3 and 6).If searches of further inventions aredesired, Applicant should identify specifically those inventions to be examined.

and it considers that the International Application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below:

Composition Claims 1, II-IX, and XII-XIX lack unity of invention under Rule 13.2 because a "special technical feature" is lacking. The sub-inventions within Group I(claim 1), and therefore the inventions of Groups II-IX, and XI-XVIII, lack a common property or activity, (PCT Rules, Annex B, Part I(f)(i)(A)) and also lack a "significant structural element" (emphasis added) PCT Rules, Annex B, Part I(f)(i)(B)(1). A structural element is significant if it "occupies a large portion of their structures, or in case the compounds have in common only a small portion of their structures, the commonly shared structure constitutes a structurally distinctive portion in view of existing prior art"(PCT Rules, Annex B, and Part I(f)(ii). Thus the peptide mimetics are most like peptides, and share few properties with the nucelotide-, carbohydrate-, pharmaceutical-, polymer-, substrate-, chiral-, or lipid-compositions.

Groups X and XI are drawn to two distinct processes of making two distinct chiral compositions, neither one of said processes (or products) suggests the other.

Comparison of the formula of Group IX, claim 26, with those of Group X, claim 33, and of Group XI, claim 36, shows that the processes are directed to preparing compositions other than that claimed, so unity of invention is absent.